St. Jude Children's Research Hospital | DNB Bioinformatics core

Single-cell ATAC-seq workflow

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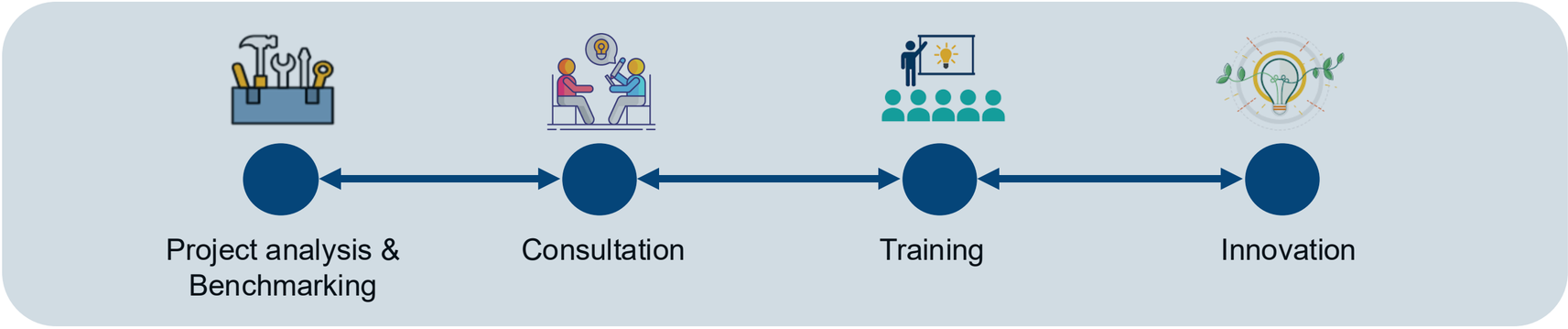
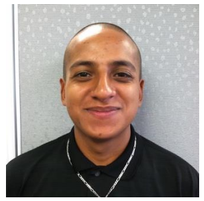
Senior Bioinformatics Research Scientist

October 29, 2025

Bioinformatics core, Department of Developmental Neurobiology

Providing advanced bioinformatic services for investigators to leverage omics data





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Senior Bioinformatics Research Scientist
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Senior Bioinformatics Research Scientist
New York, New York

Sharon Freshour, PhD
Bioinformatics Research Scientist
Tacoma, Washington

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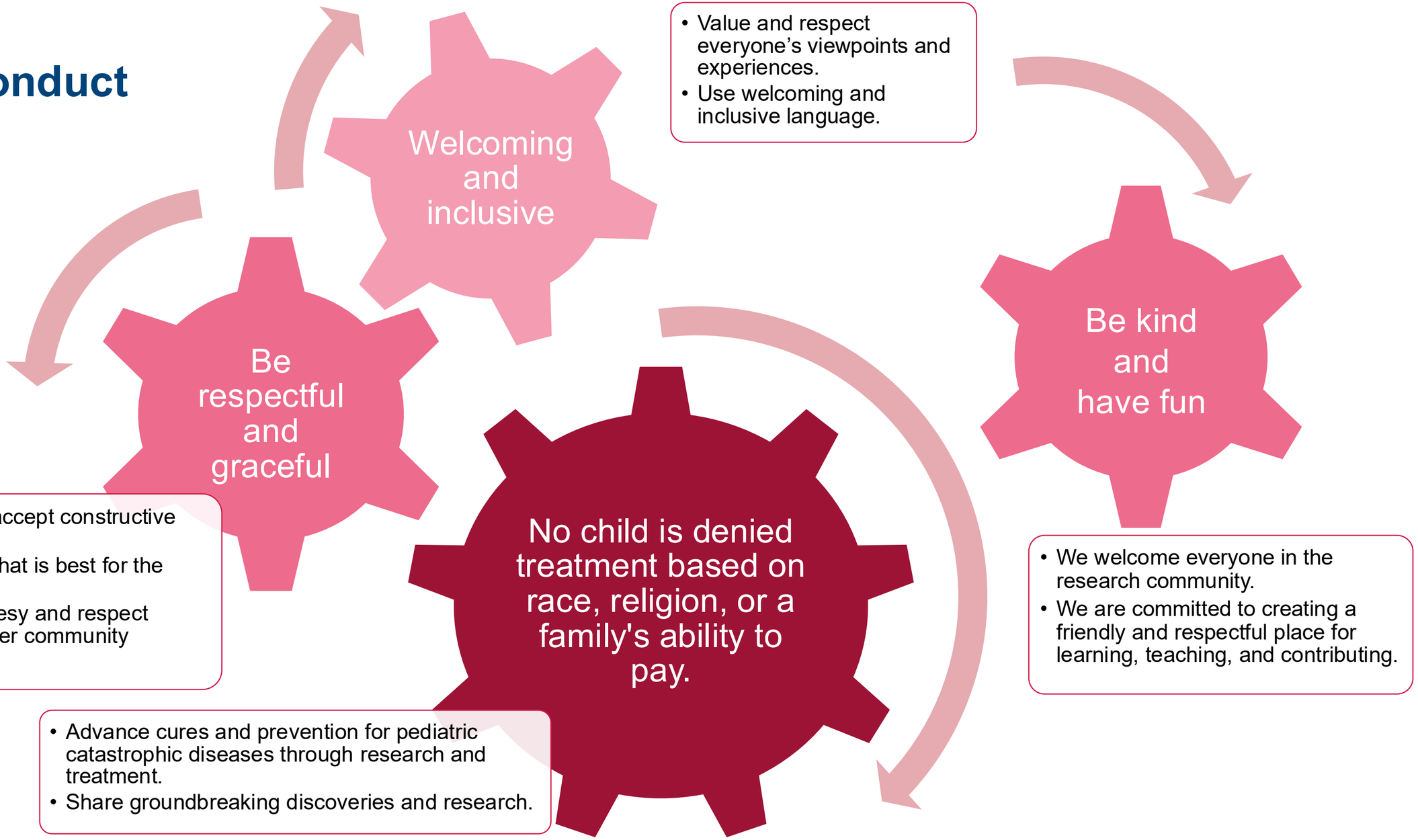
Indianapolis, Indiana

Our Tools & Resources

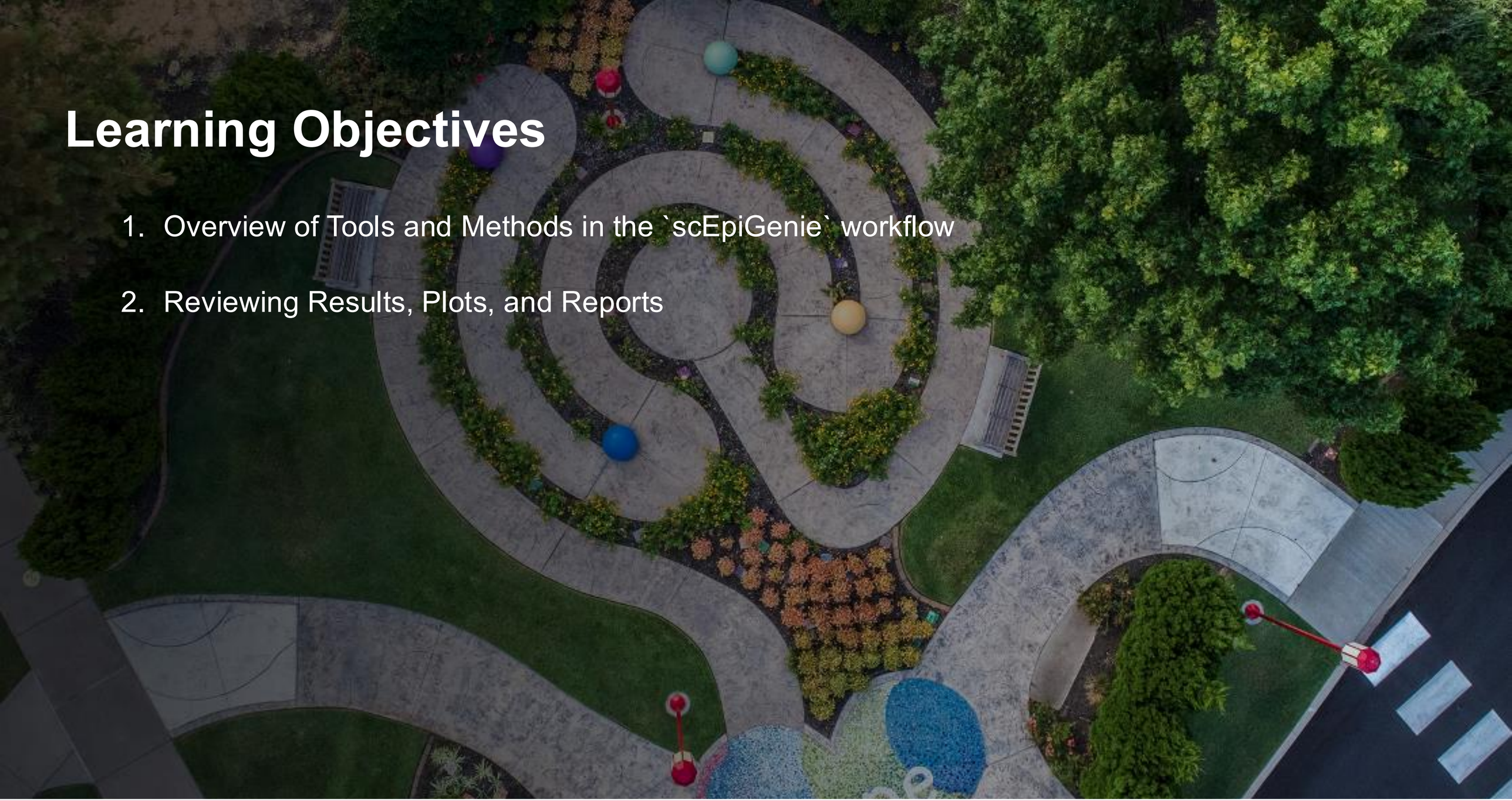
Tool/Resource	Description	Icon
Snap	sc/snRNA-Seq 10x; supports human, mouse, and dual genomes	
Snap-10x-Flex	sc/snRNA-Seq 10x Flex; supports human and mouse genomes	
sc-epigenie	scATAC-Seq 10x Flex; supports human and mouse genomes	
sc-atac-seq-bed-builder	Generate blacklist/promoter/enhancer BED files for analysis	
DevOps Containers	Reusable, independent container images for scRNA/ATAC-seq workflows	
Trainings & Workshops	Hands-on training courses and pipeline tutorials	

Code of Conduct



Learning Objectives

1. Overview of Tools and Methods in the `scEpiGenie` workflow
2. Reviewing Results, Plots, and Reports



Single cell ATAC Seq Epigenie workflow (scEpiGenie)

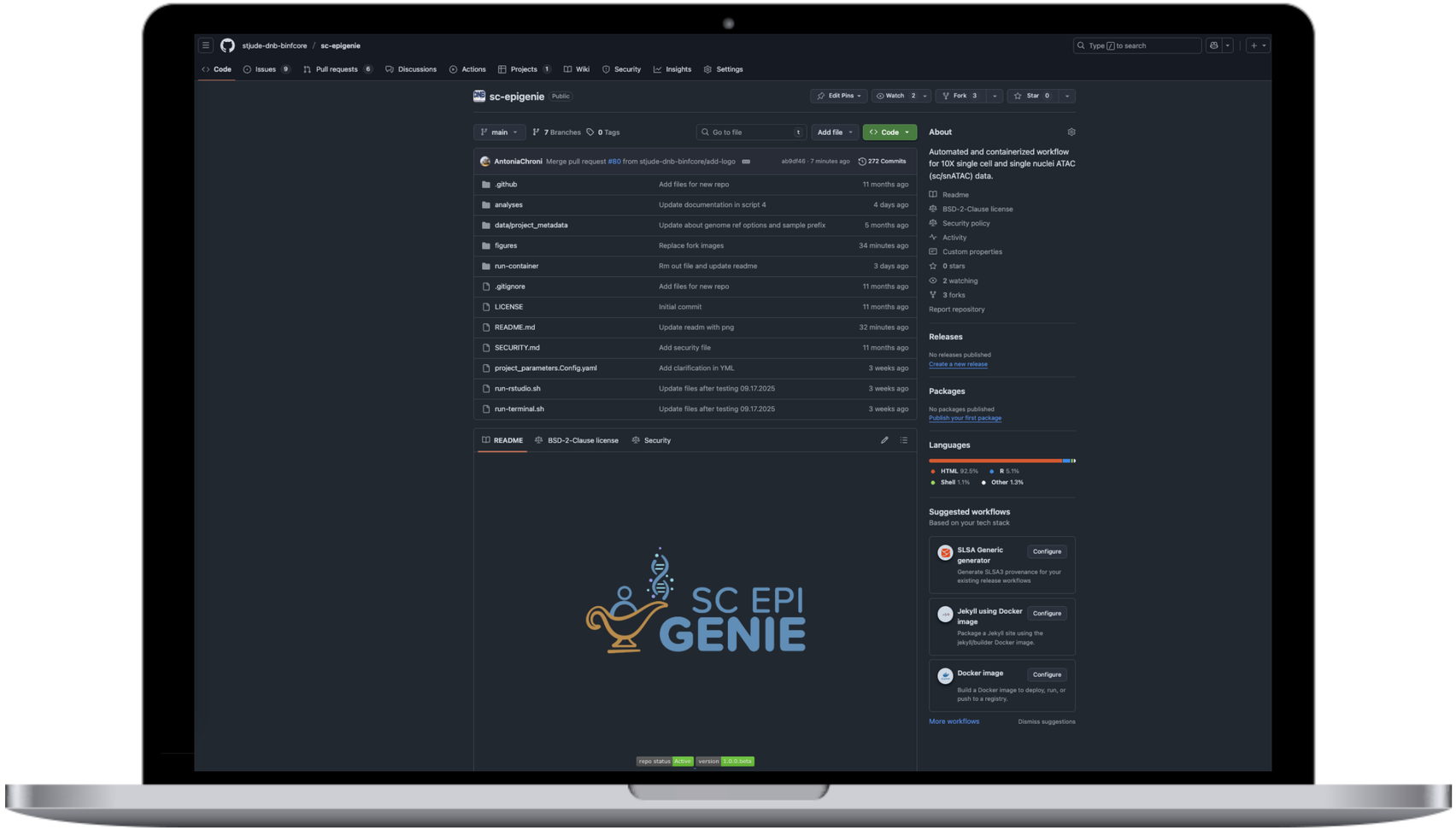
- 🚀 Automated suite of analysis modules for upstream and downstream analyses
- 🧬 scATAC data analysis from 10X sequencing technology
- 🌮 Human and mouse genome experiments
- 📝 Thorough and step-by-step documentation on how to use each analysis module
- 🐳 Reproducible and replicable results via Docker/singularity
- Publicly available at [stjude-dnb-binfcore/sc-epigenie]

scEpiGenie: A Companion to Snap

- scEpiGenie is a companion pipeline to Snap, designed for matched scRNA-seq data analysis.
- Snap pipeline is publicly available at GitHub:

stjude-dnb-binfcore/sc-rna-seq-snap

Single cell ATAC Seq Epigenie workflow (scEpiGenie)



https://github.com/stjude-dnb-binfcore/sc-epigenie

How to access the code

README License Security

For user convenience, an example project_metadata.tsv file is provided.

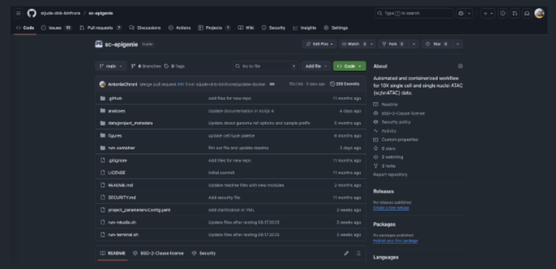
How to Use the Repository

Accessing the Code

We recommend that users fork the `sc-epigenie` repository and then clone their forked repository to their local machine. Team members should use the stjude-dnb-binfcore account, while others can use their preferred GitHub account. We welcome collaborations, so please feel free to reach out if you're interested in being added to the `stjude-dnb-binfcore` account.

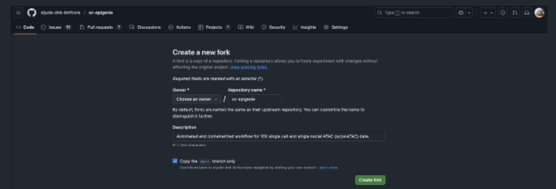
1. Fork the repository

Navigate to the main page of the `stjude-dnb-binfcore/sc-epigenie` repository and click the "Fork" button.



2. Create Your Fork

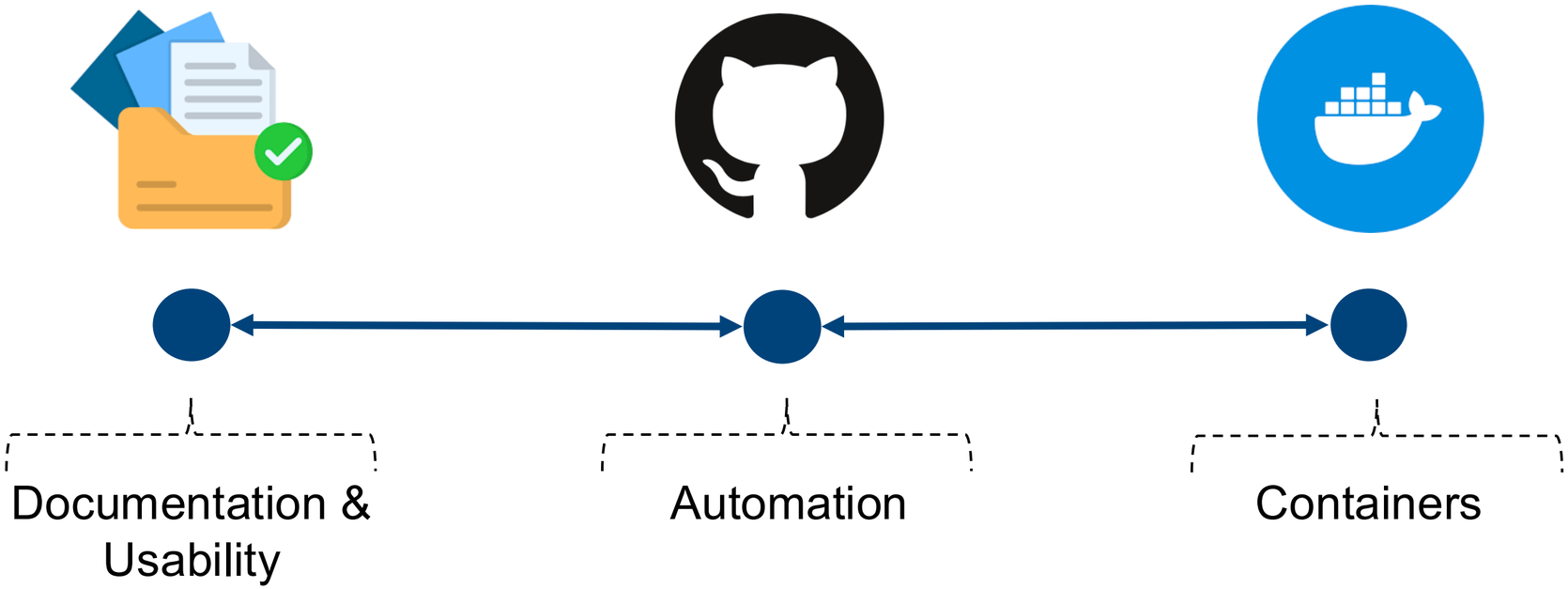
You can change the name of the forked repository (optional - unless you will use it for multiple projects). Click "Create fork" to proceed.



3. Enjoy your new project repo!

scEpiGenie is automated, reproducible and replicable

scEpiGenie is automated, reproducible and replicable

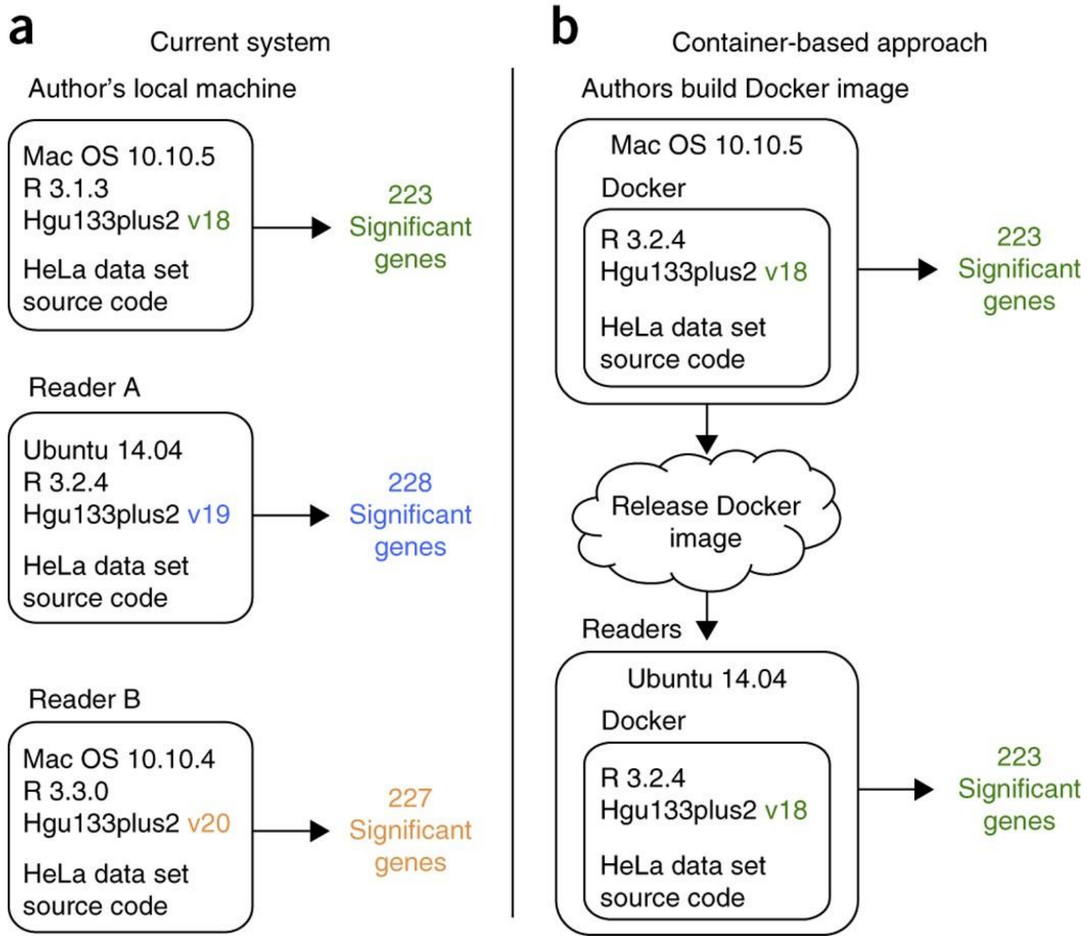


Slide from the "Automation and Reproducibility in Computational Biology" course

scEpiGenie is automated, reproducible and replicable

- Docker and Singularity
  - “Containers” that include everything from the operating system up
  - Run one OS inside another, with all the things frozen to particular versions.

- Docker image
  - achronistjude/singlecell-r4.4-seurat4.4-signac1.16



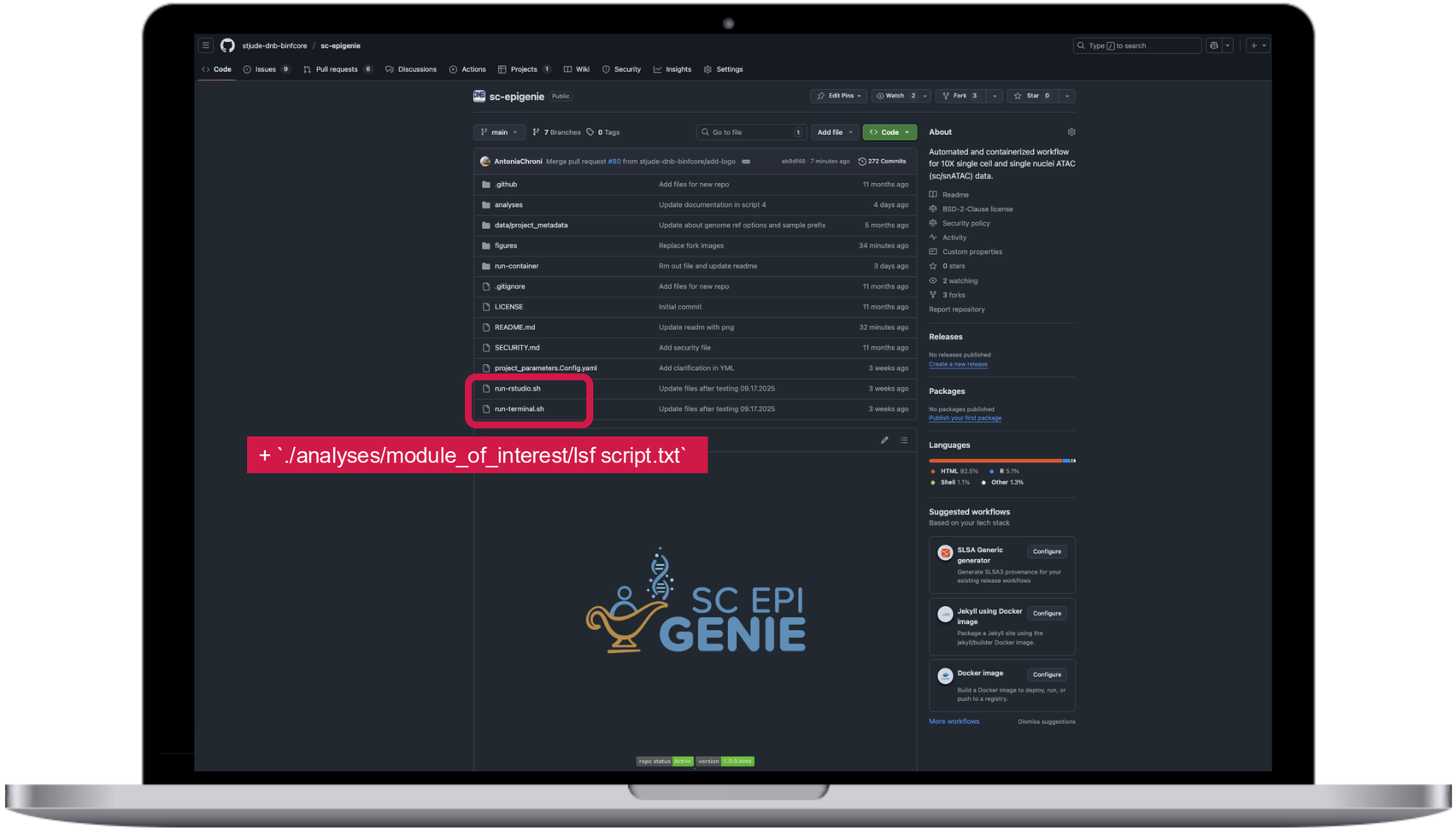
Slide from the ”Automation and Reproducibility in Computational Biology” course

DevOps Containers

- A collection of reusable container images designed to support various stages of data processing pipelines, i.e., scRNA-seq and scATAC-seq
- Each container is self-contained and portable, making it easy to use them independently in other projects or custom workflows.
- DevOps Containers is publicly available at GitHub:

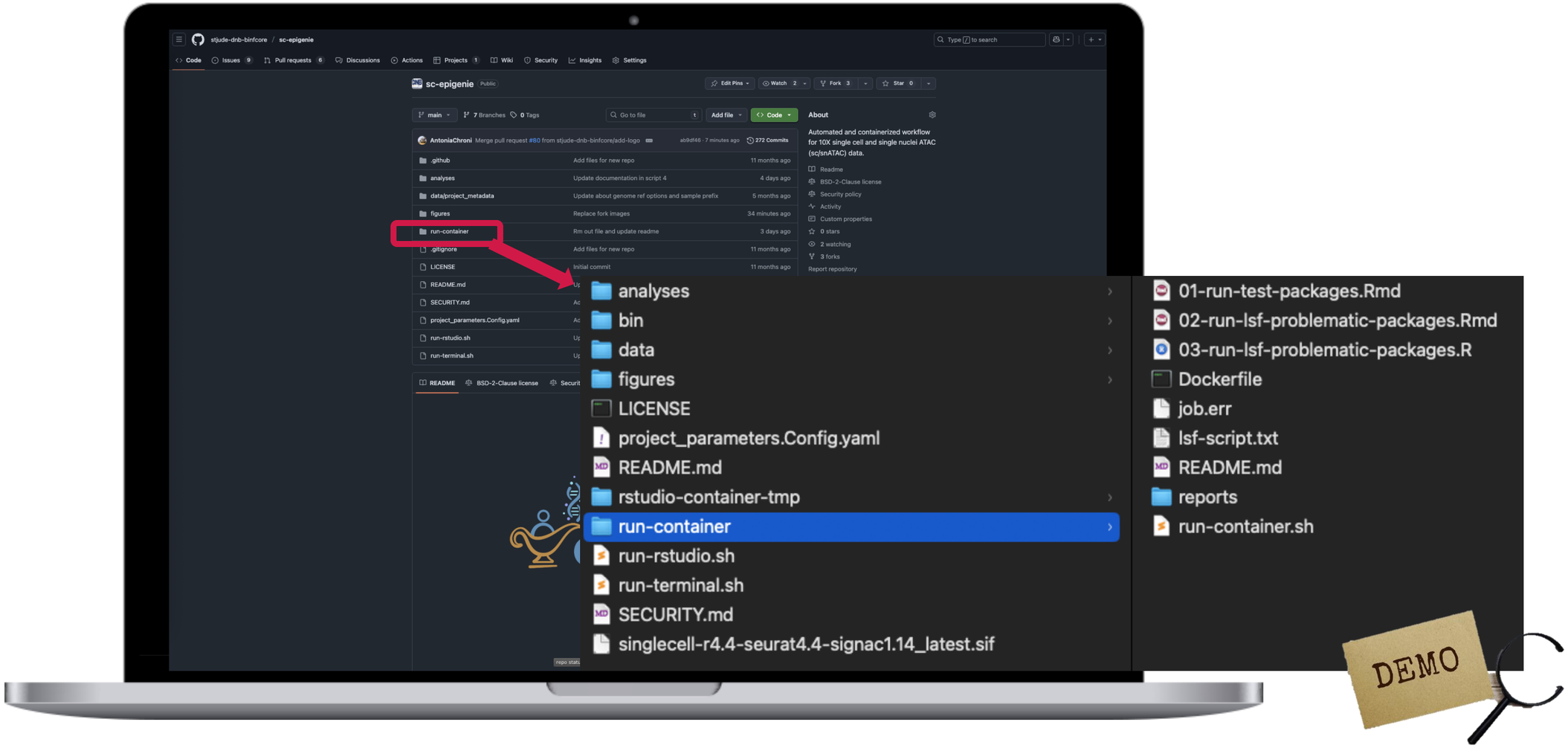
  stjude-dnb-binfcore/devops-containers

scEpiGenie is automated, reproducible and replicable



https://github.com/stjude-dnb-binfcore/sc-epigenie

scEpiGenie is automated, reproducible and replicable

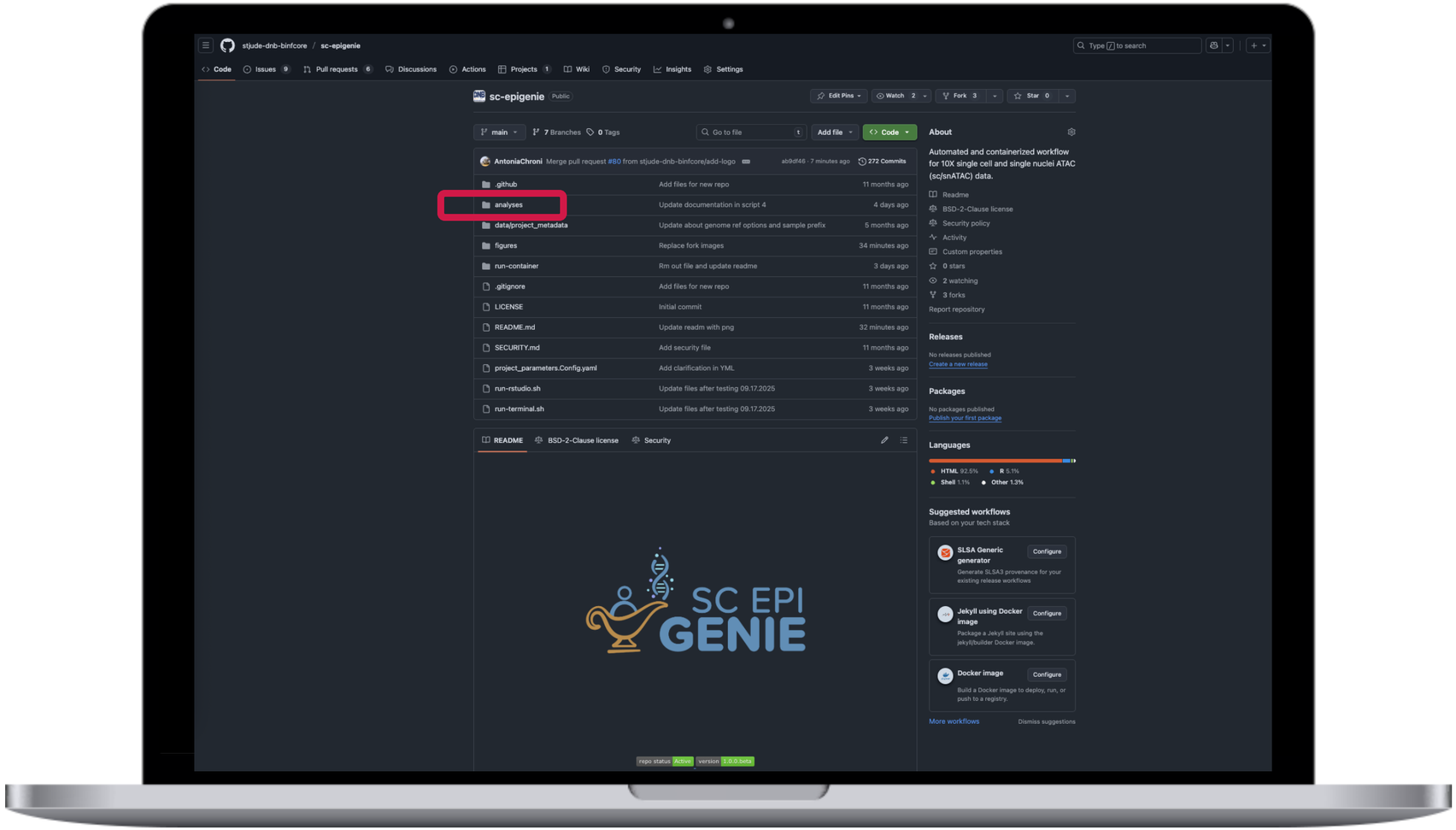


Repository organization and project tidiness

- **Use Folders/Directories**
- **Keep separate projects separated**
- **Separate sections for units within a project**
  - analyses/<module_name>
    - code
    - plots
    - results
  - data
  - figures
  - LICENSE
  - project_parameters.Config.yaml
  - SECURITY.md
  - …
- **Documentation throughout:** Describe what files do and how they are organized/folder.

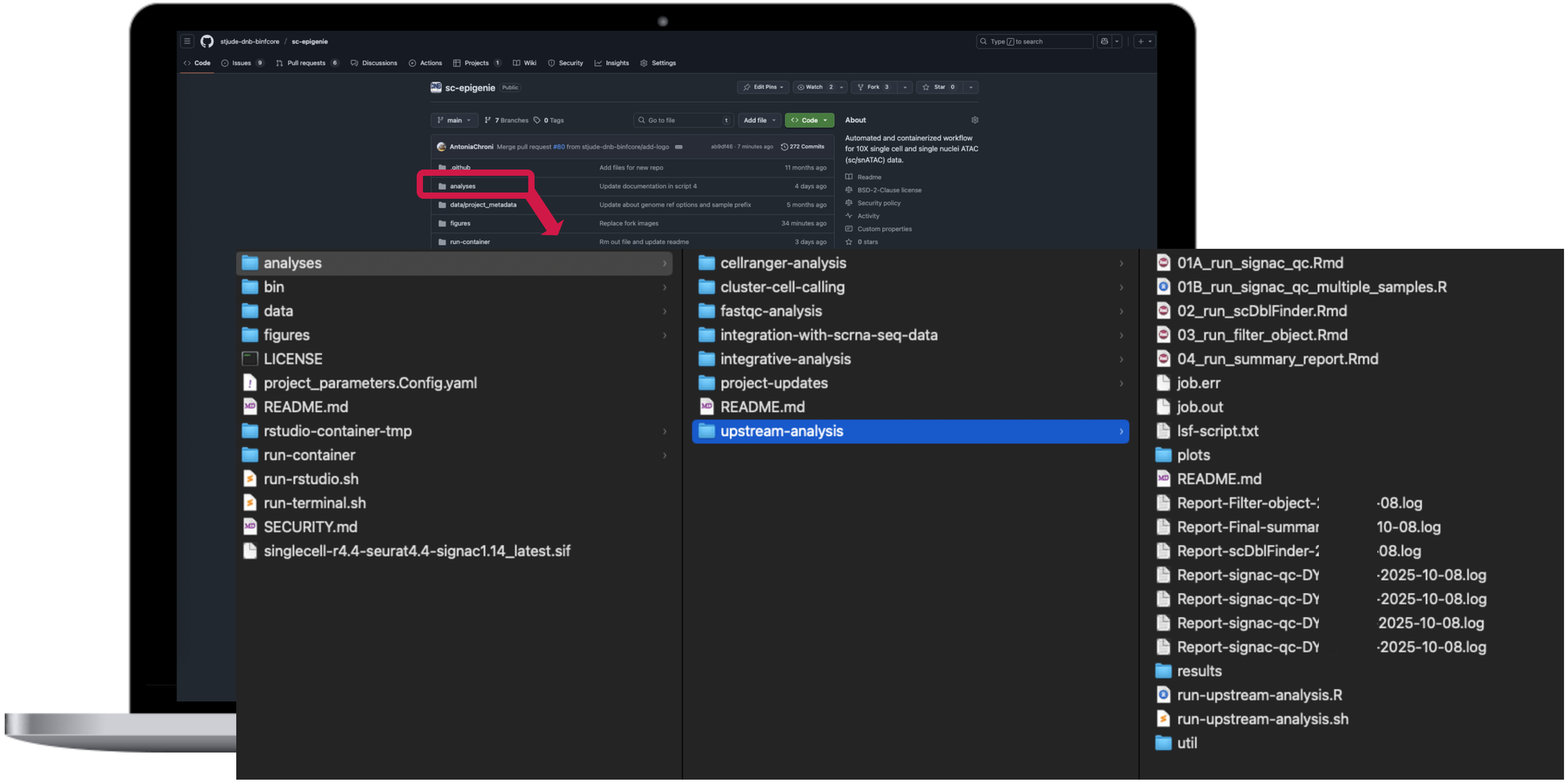
Slide from the "Automation and Reproducibility in Computational Biology" course

Repository organization and project tidiness

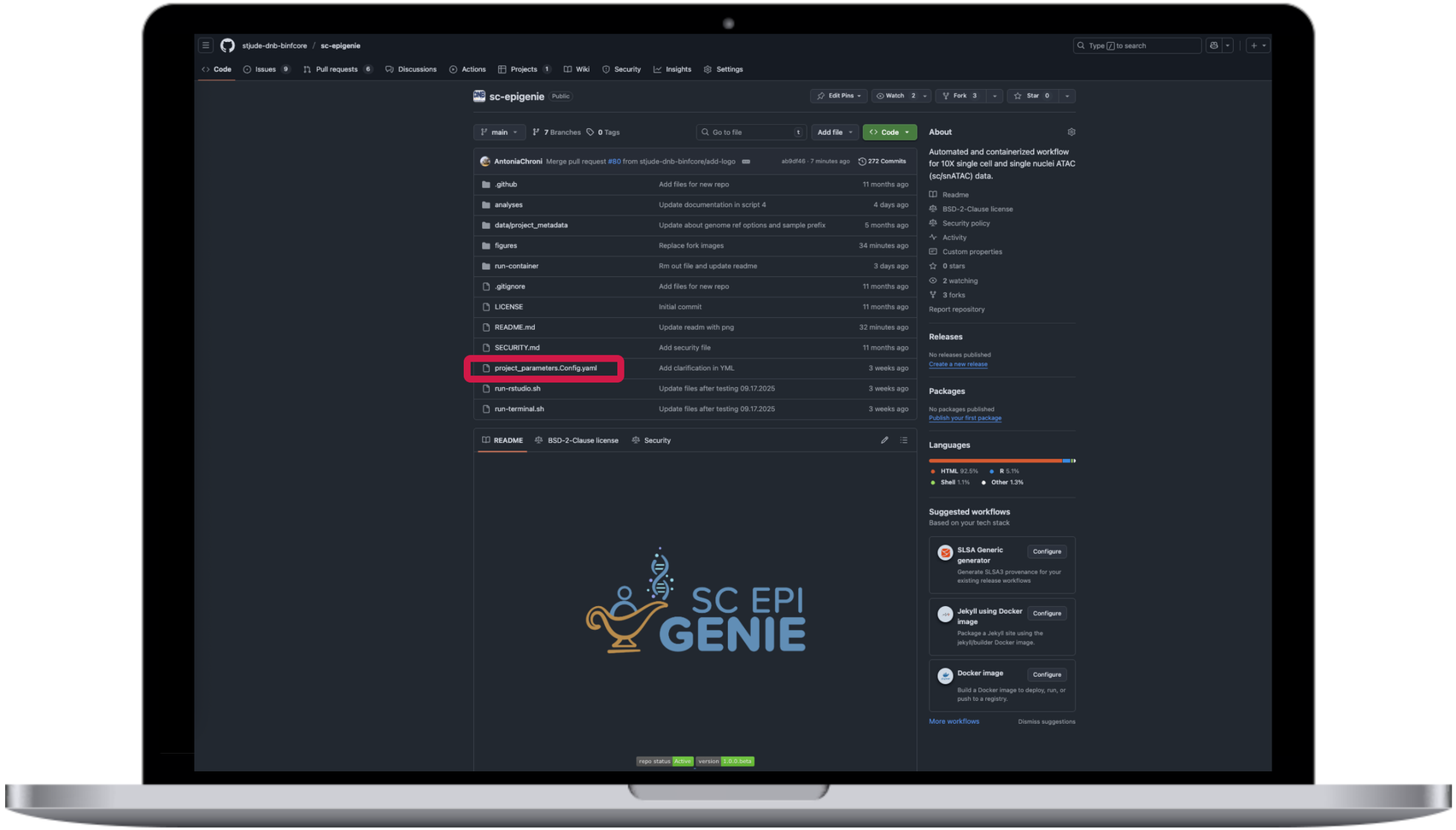


https://github.com/stjude-dnb-binfcore/sc-epigenie

My typical project organization system



Customize your project based on the experiment!



https://github.com/stjude-dnb-binfcore/sc-epigenie

Project parameters file

project_parameters.Config.yaml

```yaml
# the following parameters are the same across the project and might be needed in more than one module #
root_dir: "/sc-epigenie" # Absolute path to the main dir of the project where GitHub repo lives
data_dir: "/sc-epigenie/analyses/cellranger-analysis/results/02_cellranger_count/DefaultParameters" # Absolute path to data dir of the project with CellRanger output results. Options: "DefaultParameters", "For
metadata_dir: "/data/project_metadata" # Absolute path to metadata dir of the project.
metadata_file: "project_metadata.tsv" # Options: "project_metadata.tsv" (default) or name as user wants. It needs to be in `tsv` format. It can include one or more samples, as long as it contains at least the
genome_name: "GRCm39" # define genome reference and versioning. Options: (1) human: "GRCh38" and "hg19"; and (2) mouse: "GRCm39", "mm10", and "mm9".
PROJECT_NAME: "Victoria_Knockout - Testing cohort"
PI_NAME: "Michael A. Dyer, PhD"
TASK_ID: "NA"
PROJECT_LEAD_NAME: "NA"
DEPARTMENT: "Developmental Neurobiology"
LEAD_ANALYSTS: "Antonia Chroni, PhD"
GROUP_LEAD: "Cody A. Ramirez, PhD"
CONTACT_EMAIL: "antonia.chroni@stjude.org"
PIPELINE: "Standard scATAC-Seq Analysis in 10X Genomics data"
START_DATE: "NA"
COMPLETION_DATE: "ONGOING"

# the following parameters are set up as default values and/or are specific for the following modules:
# `./analyses/fastqc-analysis`
# FASTQ paths to the fastqc files with format: `path1/*R1-R3*.fastq.gz` are extracted from the `metadata_dir`.
# No need to manually define variables

# `./analyses/cellranger-analysis`
genome_reference_path: "./reference_genomes/cellranger-sc-atac-seq/2020-A/mus_musculus/mm10/downloads/refdata-cellranger-arc-mm10-2020-A-2.0.0" # Absolute path to genome reference to be used for the `cellrange
cellranger_parameters: "DefaultParameters" # Options: "DefaultParameters", "ForcedCells8000Parameters", or else.
genome_name_cellranger: "GRCm39" #please define the genome of preference for dual genomes. In case for single genomes, please use the same as used for `genome_name`.
# Define the sample ID prefix(es) used in this project.
# Sample IDs should follow a format like: PREFIX001 (e.g., DYE001, ABC002).
# You can specify multiple prefixes if your project uses more than one.
sample_prefix:
  - DYE
  - ABC-
  - XYZ_

# `./analyses/upstream-analysis`
genome_name_upstream: "GRCm39" # Options: (1) human: "GRCh38" and "hg19"; and (2) mouse: "GRCm39", "mm10", and "mm9".
assay_signac_qc: "peaks" # Options: "peaks" (default ALWAYS)
# Genome-to-Ensembl Mapping Options: (1) human: "GRCh38" -> "EnsDb.Hsapiens.v86"(older); "EnsDb.Hsapiens.v98" | "hg19" -> "EnsDb.Hsapiens.v75"
#                                    (2) mouse: "GRCm39" -> "EnsDb.Mmusculus.v104" (or "EnsDb.Mmusculus.v105"), | "mm10" -> "EnsDb.Mmusculus.v79" and  | "mm9" -> No official `EnsDb` package, use "TxDb.Mmusculu
annotation_Ensembl_upstream: "EnsDb.Mmusculus.v104"
species_upstream: "Mus musculus" # Options: "Hsapiens"; "Mus musculus"
object_species_upstream: "AH95775" #  # Reference genome annotation ID. Options: (1) human: "GRCh38" -> "AH75011 | "AH14502"
#                                                                                 (2) mouse: "GRCm39" -> AH95775"
min.cutoff_value_upstream: "q0" # Options: e.g., "q0" to use all peaks; "q75" to use the top 25% all peaks; "q95" to use the top 5% all peaks and so on.
#nCount_peaks_min_upstream: "100" # Total counts across all peaks Optional; can correlate with fragments
#nCount_peaks_max_upstream: "20000" # Total counts across all peaks Optional; can correlate with fragments
#nFeature_peaks_min_upstream: "500" # Number of peaks with non-zero counts  Optional
#nFeature_peaks_max_upstream: "6000" # Number of peaks with non-zero counts Optional
#peak_region_fragments_min_upstream: "100" # Total fragments in called peak regions > 3000
#peak_region_fragments_max_upstream: "20000" #"20000"
pct_reads_in_peaks_value_upstream: "20" # % of reads in peaks (signal-to-noise) > 15 or > 20
blacklist_ratio_value_upstream: "0.05" # Fraction of reads in ENCODE blacklist regions (noise metric) < 0.05
#duplicate_value_upstream: "8000" # Duplication rate  Use only if computed
#mitochondrial_value_upstream: "5" # % of mitochondrial reads < 5
TSS.enrichment_value_upstream: "4" # Transcription start site enrichment score  > 2
nucleosome_signal_value_upstream: "3" # Nucleosome periodicity signal < 4
use_threshold_filtering_upstream: "YES" # Options: "YES" (default)  or "NO" (if user prefers to use percentile filtering)
condition_value1: "condition" # Use discrete values. Min #conditions per project: 1 and Max #conditions per project: 3. To use for visualization purposes and split UMAPs. Value to be extracted from column name
condition_value2: NULL # Use discrete values. Min #conditions per project: 1 and Max #conditions per project: 3. To use for visualization purposes and split UMAPs. Value to be extracted from column name in `pr
condition_value3: NULL # Use discrete values. Min #conditions per project: 1 and Max #conditions per project: 3. To use for visualization purposes and split UMAPs. Value to be extracted from column name in `pr
print_pdf_seurat_multiple_samples: "YES" # Options: "YES" (default ALWAYS), for `018_run_signac_qc_multiple_samples.R`
use_condition_split_seurat_multiple_samples: "NO" # Options: "NO" (default ALWAYS), for  `018_run_signac_qc_multiple_samples.R`
grouping: "orig.ident" # define grouping to use
use_condition_split_filter_object: "YES" # Options: "YES" (default) or "NO", for `03_run_filter_object.Rmd`
print_pdf_filter_object: "NO" # # Options: "NO" (default ALWAYS), for `03_run_filter_object.Rmd`
use_scDblFinder_filtering_filter_object: "NO" # Options: "YES" or "NO" (default) , for `03_run_filter_object.Rmd`.
filename_filter_object_value: NULL # Options: NULL (default) or name if multiple methods are integrated and explored: "-without-scDblFinder" or "-with-scDblFinder"
#PCA_Feature_List_value: transcription.factor.gene.list # set for 04_run_filter_object.Rmd if necessary
filename_summary_report_value: NULL # Options: NULL (default) or name if multiple methods are integrated and explored: "-without-scDblFinder" or "-with-scDblFinder"
use_scDblFinder_filtering_summary_report: "NO" # Options: "YES" or "NO" (default) , for `05_run_summary_report.Rmd`.

# `./analyses/integrative-analysis`
```

How to run the code

README BSD-2-Clause license Security

Running the Code

1. Configure Your Parameters

Replace the `project_parameters.Config.yaml` file with your own file paths and parameters.

2. Navigate to an Analysis Module

Change to the relevant directory and run the desired shell script:

```
cd ./sc-epigenie/analyses/<module_of_interest>
```

3. Sync Your Fork

User needs to ensure that the main branch of the forked repository is always up to date with `stjude-dnb-binfcore/sc-epigenie:main`.

If your fork is behind the main repository (`stjude-dnb-binfcore/sc-epigenie:main`), sync it to ensure you have the latest updates. This will update the main branch of your project repo with the new code and modules (if any). This will add code and not break any analyses already run in your project repo.

When syncing your forked repository with the main repository, please be cautious of any changes made to the following files, as they are typically modified and specified for project data analysis:

- `project_parameters.Config.yaml`

Before pulling the latest changes, stash any modifications you have made to these files. This ensures that you won't accidentally overwrite your changes when syncing with the main repository.

Some useful git commands:

```
git branch
git checkout main
git config pull.rebase false

git status
git add project_parameters.Config.yaml
git commit -m "Update yaml"
```

Finally, `git pull` to get the most updated changes and code in your project repo. Please be mindful of any local changes in files in your project repo that you have done, e.g., `project_parameters.Config.yaml`. You will need to commit or stash (or restore) the changes to the yaml before completing the pull.

```
git pull
```

scEpiGenie analysis modules

README.md

Preview on Save | Preview | Run

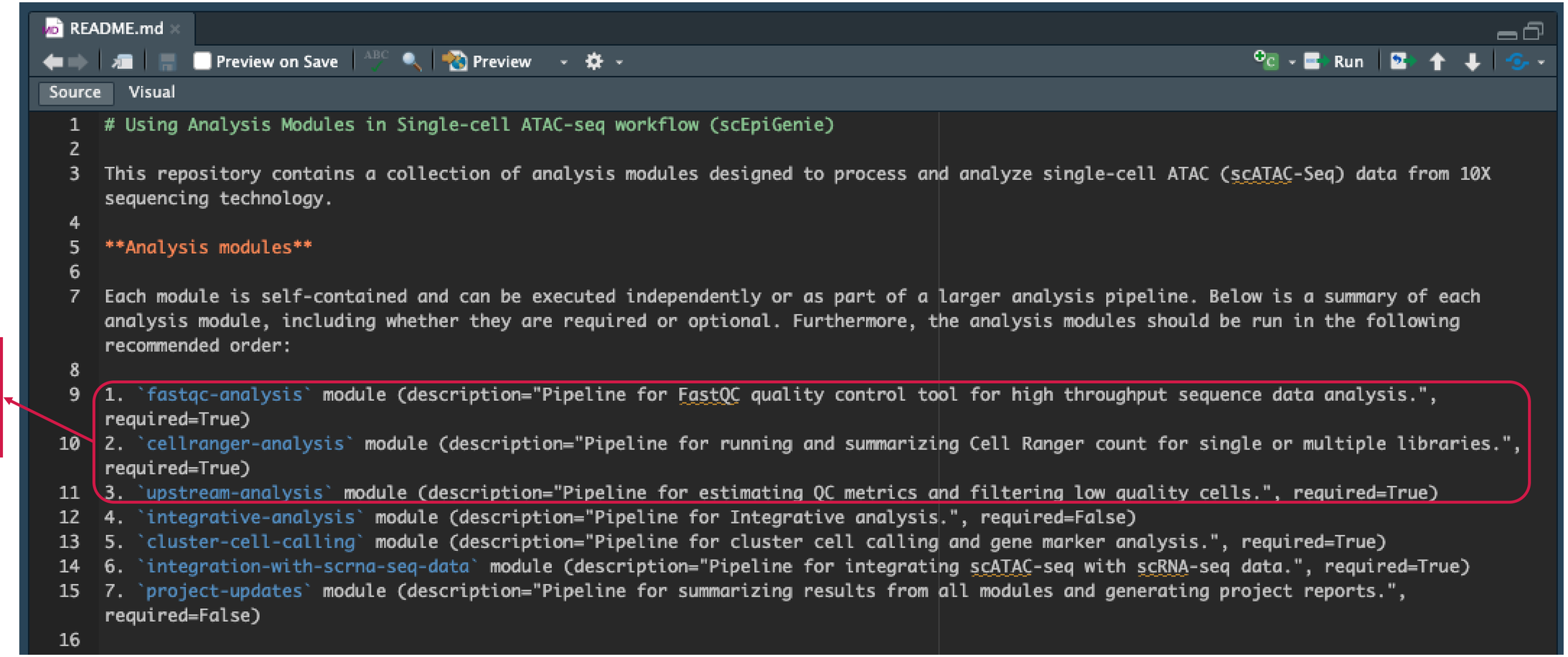
Source | Visual

```
1  # Using Analysis Modules in Single-cell ATAC-seq workflow (scEpiGenie)
2
3  This repository contains a collection of analysis modules designed to process and analyze single-cell ATAC (scATAC-Seq) data from 10X
   sequencing technology.
4
5  **Analysis modules**
6
7  Each module is self-contained and can be executed independently or as part of a larger analysis pipeline. Below is a summary of each
   analysis module, including whether they are required or optional. Furthermore, the analysis modules should be run in the following
   recommended order:
8
9  1. `fastqc-analysis` module (description="Pipeline for FastQC quality control tool for high throughput sequence data analysis.",
   required=True)
10 2. `cellranger-analysis` module (description="Pipeline for running and summarizing Cell Ranger count for single or multiple libraries.",
   required=True)
11 3. `upstream-analysis` module (description="Pipeline for estimating QC metrics and filtering low quality cells.", required=True)
12 4. `integrative-analysis` module (description="Pipeline for Integrative analysis.", required=False)
13 5. `cluster-cell-calling` module (description="Pipeline for cluster cell calling and gene marker analysis.", required=True)
14 6. `integration-with-scrna-seq-data` module (description="Pipeline for integrating scATAC-seq with scRNA-seq data.", required=True)
15 7. `project-updates` module (description="Pipeline for summarizing results from all modules and generating project reports.",
   required=False)
16
```

Upstream

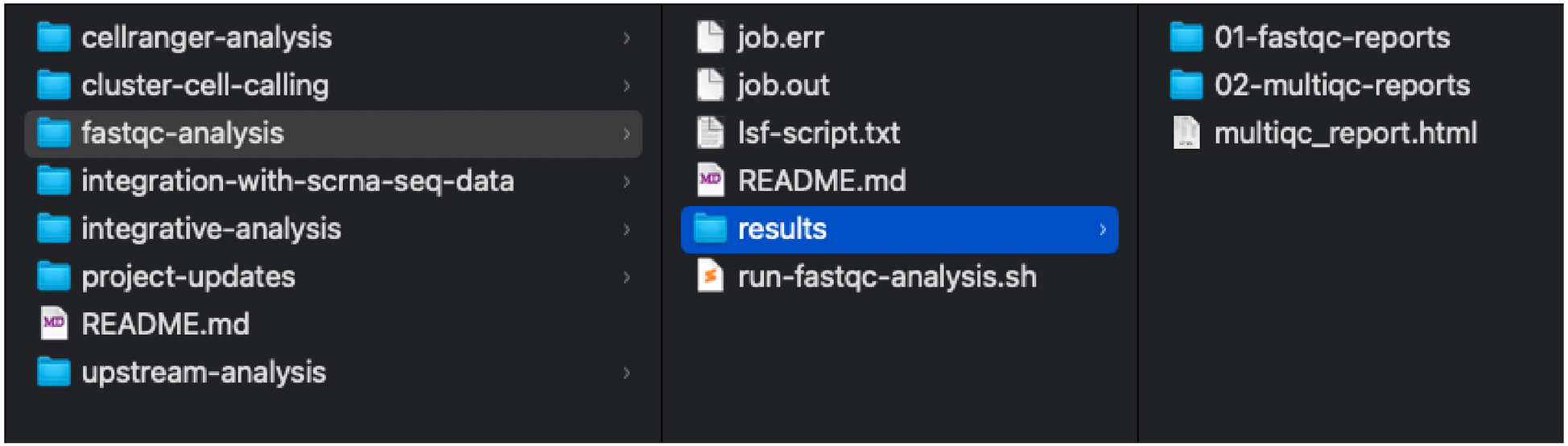
Downstream

scEpiGenie analysis modules



`fastqc-analysis` module

description="Pipeline for FastQC quality control tool for high throughput sequence data analysis.", required=True



`run-fastqc-analysis.sh`

run-fastqc-analysis.sh

```
#!/bin/bash

set -e
set -o pipefail

# set up running directory
cd "$(dirname "${BASH_SOURCE[0]}")"

######################################################################
# Read metadata_dir from YAML configuration file
metadata_dir=$(grep 'metadata_dir:' ../../project_parameters.Config.yaml | awk '{print $2}')
metadata_dir=${metadata_dir//\"/}  # Removes all double quotes
echo "Metadata directory: $metadata_dir"  # Output the directory path

# Define the path to the metadata file (adjust to your actual file)
metadata_file="$metadata_dir/project_metadata.tsv"

# Check if metadata file exists
if [ ! -f "$metadata_file" ]; then
  echo "Error: Metadata file '$metadata_file' does not exist."
  exit 1
fi

######################################################################
# Define the column name for FASTQ - this is by default as defined in the `project_metadata.tsv` file
fastq_column_name="FASTQ"

# Extract the column number of FASTQ from the header (first line)
column_number=$(head -n 1 "$metadata_file" | tr '\t' '\n' | grep -n "^$fastq_column_name$" | cut -d: -f1)

# Check if the FASTQ column exists
if [ -z "$column_number" ]; then
  echo "Error: Column '$fastq_column_name' not found in the metadata file."
  exit 1
fi

######################################################################
# Declare an array to store the FASTQ values
declare -a fastqc_dir

# Use awk to extract the FASTQ column values (skip the header)
# Adjust delimiter (tab or comma) based on your file format
while IFS= read -r fastq_value; do
  fastqc_dir+=("$fastq_value")
done < <(awk -F'\t' -v col="$column_number" 'NR > 1 {print $col}' "$metadata_file")

# Check if the array has values
if [ "${#fastqc_dir[@]}" -gt 0 ]; then
  echo "fastqc_dir is an array with ${#fastqc_dir[@]} elements."
else
  echo "fastqc_dir is empty or failed to extract values."
fi

# Optionally print the FASTQ values for verification
echo "FASTQ values extracted:"
for dir in "${fastqc_dir[@]}"; do
  echo "$dir"
done

######################################################################
# Create results dir
mkdir -p results
mkdir -p results/01-fastqc-reports

######################################################################
##############################################################################################################
###### STEP 1 ###### ###### ###### ###### ###### ###### ###### ###### ###### ###### ###### ###### ######
##############################################################################################################
# Run FastQC per library

# Loop through the array and echo each element
#for dir in "${fastqc_dir_array[@]}";
for dir in $(echo "${fastqc_dir[@]}" | tr ' ' '\n' | sort);
```

`run-fastqc-analysis.sh` and `lsf-script.txt`

run-fastqc-analysis.sh

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# Check if the array has values
if [ "${#fastqc_dir[@]}" -gt 0 ]; then
  echo "fastqc_dir is an array with ${#fastqc_dir[@]} elements."
else
  echo "fastqc_dir is empty or failed to extract values."
fi

# Optionally print the FASTQ values for verification
echo "FASTQ values extracted:"
for dir in "${fastqc_dir[@]}"; do
  echo "$dir"
done

######################################################################
# Create results dir
mkdir -p results
mkdir -p results/01-fastqc-reports

######################################################################
##############################################################################################################
###### STEP 1 ###### ###### ###### ###### ###### ###### ###### ###### ###### ###### ###### ###### ######
##############################################################################################################
# Run FastQC per library

# Loop through the array and echo each element
#for dir in "${fastqc_dir_array[@]}";
for dir in $(echo "${fastqc_dir[@]}" | tr ' ' '\n' | sort);
```

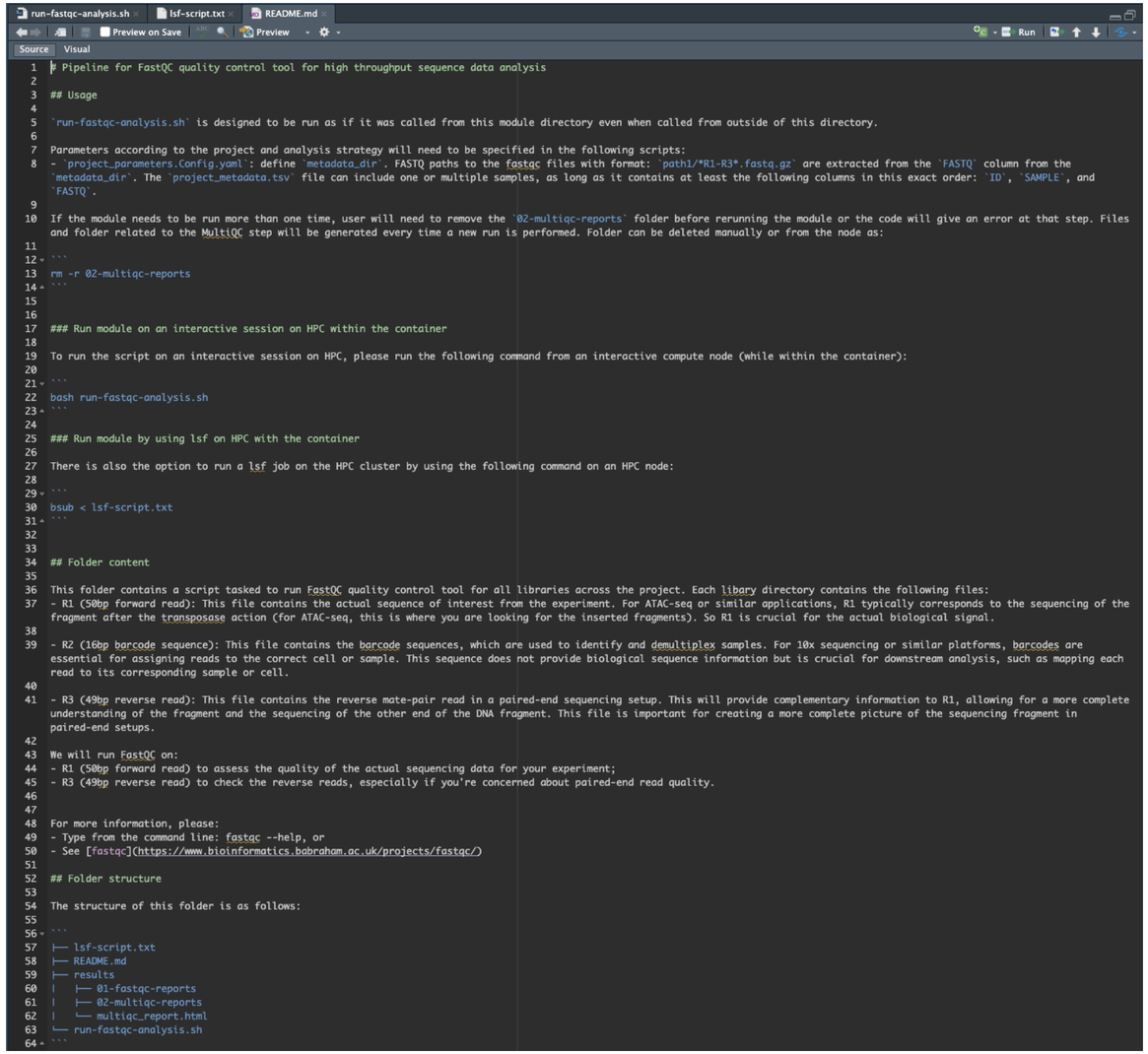
run-fastqc-analysis.sh | lsf-script.txt

```
#BSUB -P project
#BSUB -J fastqc-analysis
#BSUB -oo job.out -eo job.err
#BSUB -n 2
#BSUB -R "rusage[mem=16GB] span[hosts=1]"
#BSUB -cwd "."

singularity exec ../../singlecell-r4.4-seurat4.4-signac1.14_latest.sif bash run-fastqc-analysis.sh

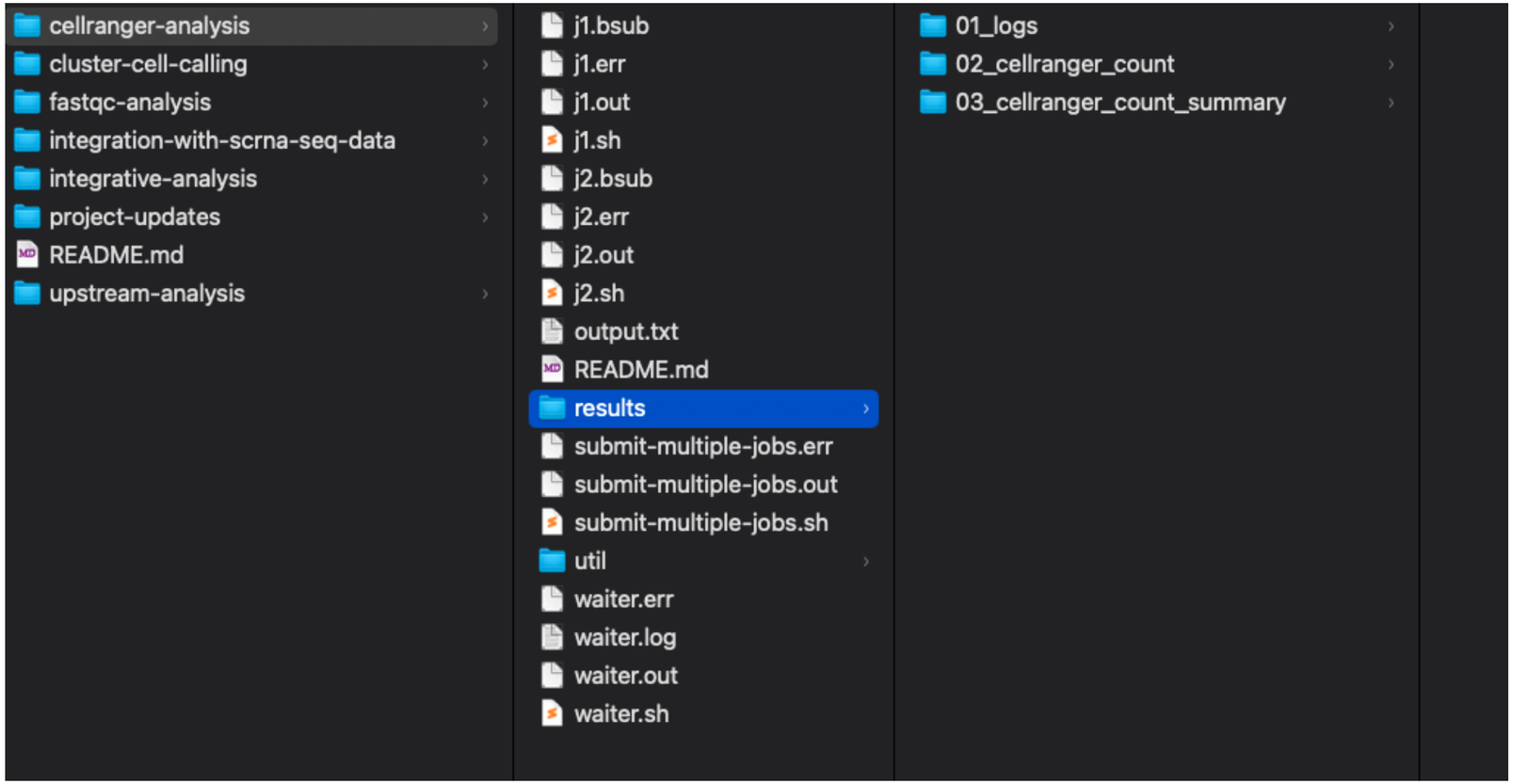
```

README.md


run-fastqc-analysis.sh
lsf-script.txt
README.md
Preview on Save
Preview
Run
Source
Visual
Pipeline for FastQC quality control tool for high throughput sequence data analysis
Usage
`run-fastqc-analysis.sh` is designed to be run as if it was called from this module directory even when called from outside of this directory.
Parameters according to the project and analysis strategy will need to be specified in the following scripts:
- `project_parameters.Config.yaml`: define `metadata_dir`. FASTQ paths to the fastqc files with format: `path1/*R1-R3*.fastq.gz` are extracted from the `FASTQ` column from the `metadata_dir`. The `project_metadata.tsv` file can include one or multiple samples, as long as it contains at least the following columns in this exact order: `ID`, `SAMPLE`, and `FASTQ`.
If the module needs to be run more than one time, user will need to remove the `02-multiqc-reports` folder before rerunning the module or the code will give an error at that step. Files and folder related to the MultiQC step will be generated every time a new run is performed. Folder can be deleted manually or from the node as:
```
rm -r 02-multiqc-reports
```
Run module on an interactive session on HPC within the container
To run the script on an interactive session on HPC, please run the following command from an interactive compute node (while within the container):
```
bash run-fastqc-analysis.sh
```
Run module by using lsf on HPC with the container
There is also the option to run a lsf job on the HPC cluster by using the following command on an HPC node:
```
bsub < lsf-script.txt
```
Folder content
This folder contains a script tasked to run FastQC quality control tool for all libraries across the project. Each libary directory contains the following files:
- R1 (50bp forward read): This file contains the actual sequence of interest from the experiment. For ATAC-seq or similar applications, R1 typically corresponds to the sequencing of the fragment after the transposase action (for ATAC-seq, this is where you are looking for the inserted fragments). So R1 is crucial for the actual biological signal.
- R2 (16bp barcode sequence): This file contains the barcode sequences, which are used to identify and demultiplex samples. For 10x sequencing or similar platforms, barcodes are essential for assigning reads to the correct cell or sample. This sequence does not provide biological sequence information but is crucial for downstream analysis, such as mapping each read to its corresponding sample or cell.
- R3 (49bp reverse read): This file contains the reverse mate-pair read in a paired-end sequencing setup. This will provide complementary information to R1, allowing for a more complete understanding of the fragment and the sequencing of the other end of the DNA fragment. This file is important for creating a more complete picture of the sequencing fragment in paired-end setups.
We will run FastQC on:
- R1 (50bp forward read) to assess the quality of the actual sequencing data for your experiment;
- R3 (49bp reverse read) to check the reverse reads, especially if you're concerned about paired-end read quality.
For more information, please:
- Type from the command line: fastqc --help, or
- See [fastqc](https://www.bioinformatics.babraham.ac.uk/projects/fastqc/)
Folder structure
The structure of this folder is as follows:
```
├── lsf-script.txt
├── README.md
├── results
|   ├── 01-fastqc-reports
|   ├── 02-multiqc-reports
|   └── multiqc_report.html
└── run-fastqc-analysis.sh
```

`cellranger-analysis` module

description="Pipeline for running and summarizing Cell Ranger count for single or multiple libraries.", required=True.



`cellranger-analysis` module

README.md

Preview on Save | Preview | Run

Source | Visual

```
# Pipeline for running and summarizing Cell Ranger count for single or multiple libraries for single-cell ATAC (scATAC-Seq) data analysis in 10X Genomics data

## Usage

`submit-multiple-jobs.sh` is designed to be run as if it was called from this module directory even when called from outside of this directory.

The `submit-multiple-jobs.sh` script is designed to run the following two steps:
   - Step 1: To run the `j1.sh` script to align single or multiple libraries in parallel, i.e., `run-cellranger-analysis`.
   - Step 2: To run `j2.sh` to summarize alignment results, i.e., `summarize-cellranger-analysis`. The latter script will be on hold and executed once all libraries are aligned and `j1.sh` is complete. This is been taken care of by `waiter.sh` script.


Parameters according to the project and analysis strategy will need to be specified in the following scripts:
- `../../project_parameters.Config.yaml`: define the `metadata_dir`, `genome_reference_path`, `cellranger_parameters`, and `genome_name_cellranger`. For a list of genome references maintained and supported by the Bioinformatics Core at DNB, please review the [Genome References wiki page](https://github.com/stjude-dnb-binfcore/sc-rna-seq-snap/wiki/2.-Genome-References). Please submit an [issue](https://github.com/stjude-dnb-binfcore/sc-epigenie/issues) to request the path to the reference genome of preference. Otherwise, specify the path to the reference genome of your preference.

User also need to define `sample_prefix` with the Sample ID used for the samples of the project. Sample IDs should follow a format like: PREFIX001 (e.g., DYE001, ABC-002, XYZ_003). You can specify multiple prefixes if your project uses more than one.

- `j1.sh`:
  - `--force-cells`: User can add flags as necessary for their analyses and compare alignments with CellRanger, e.g., by using `--force-cells=8000` to constrain the number of cells to be used for alignment. We recommend running by default and, after careful assessment, editing parameters. We have found that the default parameters set up here work well for most cases.


### Handling Top-Ups and Technical Replicates in Cell Ranger Count

If a sample (`ID` column) has multiple technical replicates (i.e., multiple sequencing runs of the same library), list all corresponding FASTQ file paths in a single row of the metadata file (`FASTQ` column), separated by commas with no spaces. Use the same format for samples with multiple library names (`SAMPLE` column). Make sure that sample names and their FASTQ paths are listed in matching order (`SAMPLE` and `FASTQ` column, respectively).


Example Metadata Format 1:

| ID | SAMPLE | FASTQ |
|:---|:---|:---|
| DYE001 | seq_submission_code1_sample1 | /absolute_path/seq_submission_code1/replicate1,/absolute_path/seq_submission_code1/replicate2 |
```

```
Example Metadata Format 2:

| ID | SAMPLE | FASTQ |
|:---|:---|:---|
| DYE001 | seq_submission_code1_sample1,seq_submission_code2_sample2 | /absolute_path/seq_submission_code1,/absolute_path/seq_submission_code2 |


Cell Ranger will automatically recognize these as multiple libraries for the same sample (`ID` column) and merge them into a single output for the sample during processing. There is no need to manually combine or rename the files–simply format them correctly in the metadata file, and the pipeline will handle the rest.


### Run module on HPC

To run all of the scripts in this module sequentially on an interactive session on HPC, please run the following command from an interactive compute node:

```
bsub < submit-multiple-jobs.sh
```

Please note that this will run the analysis module outside of the container while submitting lsf job on HPC. This is currently the only option of running the `cellranger-analysis` module. By default, we are using `python/3.9.9` and `cellranger-atac/2.1.0` as available on St Jude HPC.

Folder content

This folder contains scripts tasked to run and summarize Cell Ranger count for single or multiple libraries for single-cell ATAC (scATAC-Seq) data analysis in 10X Genomics data across the project. For more information and updates, please see [Cell Ranger support page](https://support.10xgenomics.com/single-cell-atac/software/pipelines/latest/using/count).

This module uses CellRanger-atac v2.1.0 for the alignment.

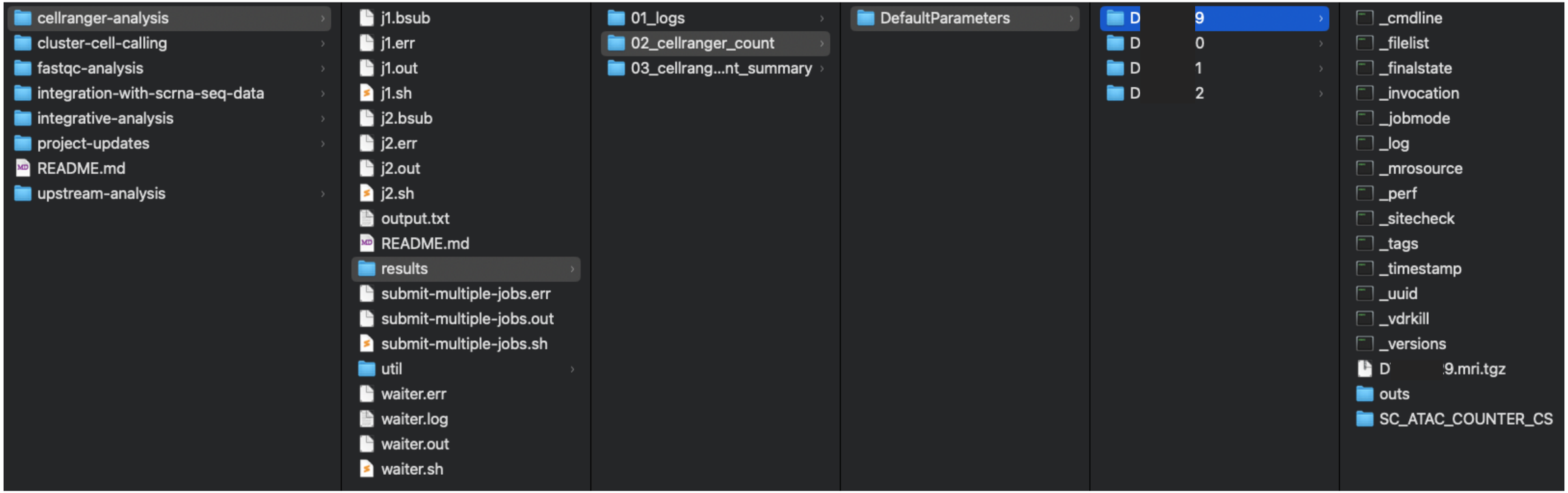
For more information, on how to review the web summary file in the output folder of the Cell Ranger ATAC analysis software, please see the [Interpreting Cell Ranger ATAC Web Summary Files for Single Cell ATAC Assay](https://www.10xgenomics.com/support/epi-atac/documentation/steps/sequencing/interpreting-cell-ranger-atac-web-summary-files-for-single-cell-atac-assay) file.

Folder structure

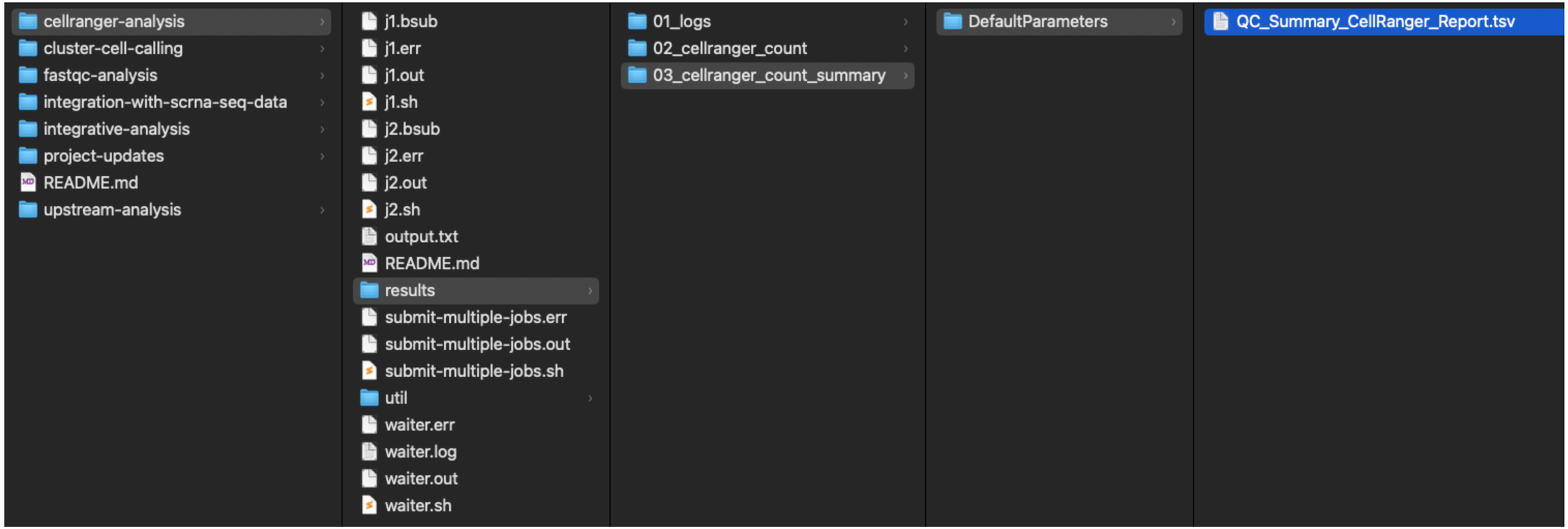
The structure of this folder is as follows:

```
├── j1.sh
├── j2.sh
├── README.md
├── results
|   ├── 01_logs
|   ├── 02_cellranger_count
|   |   └── ${cellranger_parameters}
|   └── 03_cellranger_count_summary
|       └── ${cellranger_parameters}
├── submit-multiple-jobs.sh
├── util
|   └── summarize_cellranger_results.py
└── waiter.sh
```
```

`cellranger-analysis` module



`cellranger-analysis` module

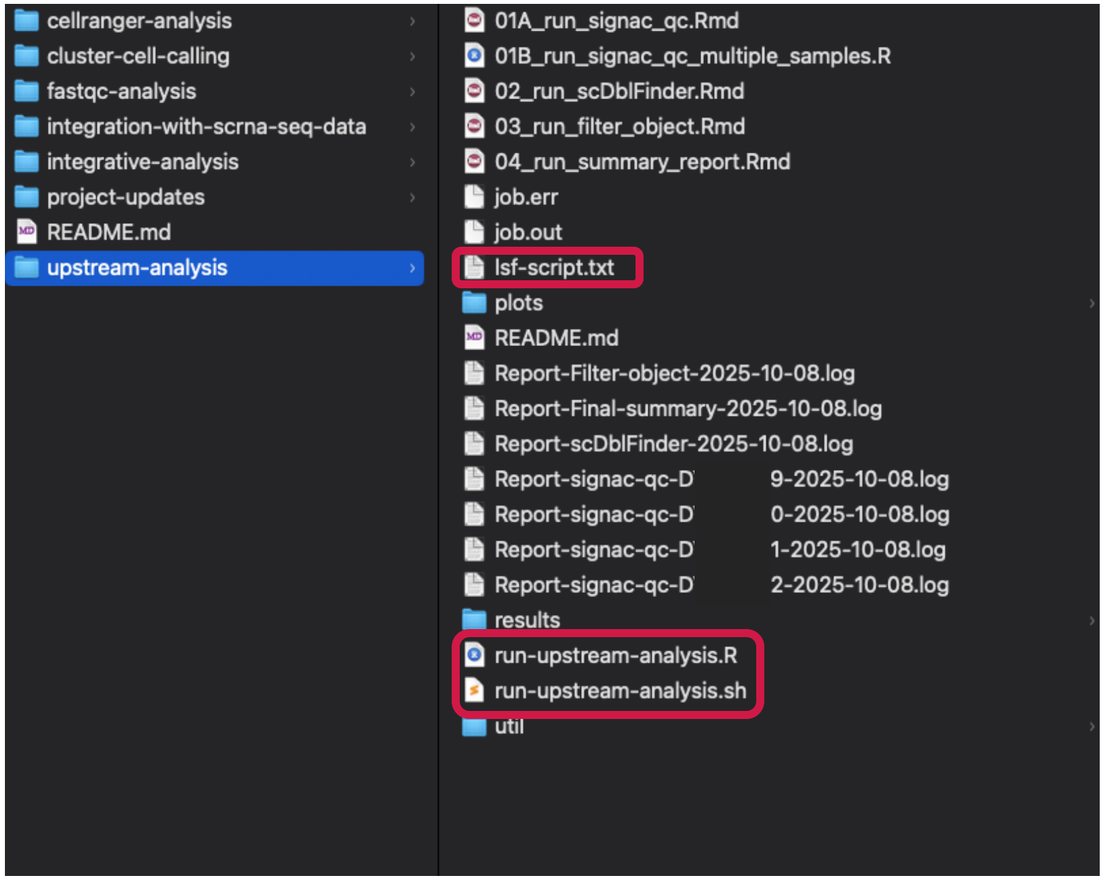


`cellranger-analysis` module

- **sc-atac-bed-builder.** 🚧 Currently under construction. Stay tuned! 🚧
  - https://github.com/stjude-dnb-binfcore/sc-atac-seq-bed-builder

`upstream-analysis` module

description="Pipeline for estimating QC metrics and filtering low quality cells.", required=True.



`upstream-analysis` module

README.md

Preview on Save | Preview | Run

Source | Visual

````markdown
# Pipeline for estimating QC metrics for sc-ATAC-Seq Analysis in 10X Genomics data

## Usage

`run-upstream-analysis.sh` is designed to be run as if it was called from this module directory even when called from outside of this directory.

Parameters according to the project and analysis strategy will need to be specified in the following scripts:
- `project_parameters.Config.yaml` located at the `root_dir`.
- `future.globals.maxSize` is hardwired coded in the `run-upstream-analysis.R`. If necessary, user can increase/decrease resources.


### Run module on an interactive session on HPC within the container

To run all of the Rscripts in this module sequentially on an interactive session on HPC, please run the following command from an interactive compute node (while within the container):

```
bash run-upstream-analysis.sh
```

### Run module by using lsf on HPC with the container

There is also the option to run a lsf job on the HPC cluster by using the following command on an HPC node:

```
bsub < lsf-script.txt
```


## Folder content

This folder contains scripts tasked to:
(1) Infer QC metrics and associated plots to visually explore the quality of each library of the project.
(2) Evaluate QC metrics and set filters to remove low quality cells in 10x single-cell-ATAC-sequencing libraries.

## QC Steps and methods

The pipeline offers flexibility for users to include or exclude methods and adjust the workflow during QC based on factors such as sequence type, expected cell number, experiment type, and genome reference.

By default, the pipeline runs all methods from steps (1-2). Step (1) is mandatory for basic QC filtering, while integration of step (2) is optional. This can be configured in the `project_parameters.Config.yaml` file. However, we recommend reviewing all results, as this can provide valuable insights into the overall quality of each library.


### (1) Signac QC metrics

[Signac](https://stuartlab.org/signac/articles/pbmc_vignette) workflow is implemented to pre-process, filter and plot the ATAC-sequencing data. For more tutorials, see [Introduction to single cell ATAC data analysis in R](https://www.youtube.com/watch?v=e2396GKFMRY&ab_channel=Sanbomics) and [How to analyze single-cell ATAC-Seq data in R | Detailed Signac Workflow Tutorial](https://www.youtube.com/watch?v=yEKZJVjc5DY&ab_channel=Bioinformagician).

The CellRanger output from the `cellranger-analysis` module will be used for this step. User will have to define `params` as needed for their experiment.
  - Calculate QC metrics: Nucleosome banding pattern, Transcriptional start site (TSS) enrichment score, Total number of fragments in peaks, Fraction of fragments in peaks, Ratio reads in genomic blacklist regions, etc.
  - Before and after filter: Plot "pct_reads_in_peaks", "peak_region_fragments", "TSS.enrichment", "blacklist_ratio", "nucleosome_signal", "nCount_peaks", "nFeature_peaks", "pct_reads_in_peaks_promoters", "pct_reads_in_peaks_enhancers", "duplicate", "mitochondrial".
  - Data are normalized by using term frequency-inverse document frequency (TF-IDF) normalization. Then, we select the top n% of features (peaks) for dimensional reduction, or remove features present in less than n cells with the [FindTopFeatures()](https://stuartlab.org/signac/reference/findtopfeatures) function. We next run singular value decomposition (SVD) on the TD-IDF matrix, using the features (peaks) selected above. This returns a reduced dimension representation of the object (for users who are more familiar with scRNA-seq, you can think of this as analogous to the output of PCA). The combined steps of TF-IDF followed by SVD are known as latent semantic indexing (LSI). After that the cells are embedded in a low-dimensional space we can run UMAP from the Seurat package.

Here, the user can select to implement the following strategies to remove low quality cells via the `run_QC` function:
- `approach 1` Filter cells based on various variables as defined in the `params`.
- `approach 2` Use percentile filtering. This will be defined in the `params` by setting `use_threshold_filtering_upstream: "NO"`.

Moreover, only libraries with more than 500 cells will be kept for merging and integration purposes. This value is a commonly used threshold for many single-cell ATAC-seq studies as a minimum for obtaining reliable and meaningful analysis.
- Statistical Power: At least 500 cells are typically needed to ensure the analysis has enough statistical power to detect meaningful biological signals.
- Cell Diversity: With fewer cells, you may not capture sufficient cellular diversity, leading to incomplete or biased results.
- Clustering: Some clustering algorithms in single-cell RNA-seq require a minimum number of cells to create robust, meaningful clusters.

### Genome-to-Ensembl Mapping Options

We need tp assign gene annotations from EnsDb based on the genome reference used for the cohort. For more information on the various Ensembl releases, you can refer to the [Table of Assemblies](https://useast.ensembl.org/info/website/archives/assembly.html) and [Build Notes for Reference Packages](https://www.10xgenomics.com/support/software/cell-ranger/downloads/cr-ref-build-steps#ref-2020-a).

| Species | Genome Build | Ensembl Version(s)        | Annotation Package                              |
|---------|-------------|-------------------------|-----------------------------------------------|
| Human   | hg19 (GRCh37) | v75                      | `EnsDb.Hsapiens.v75`                           |
| Human   | hg38 (GRCh38) | v86 (older), v98 (newer) | `EnsDb.Hsapiens.v86`, `EnsDb.Hsapiens.v98`     |
| Mouse   | mm9 (NCBI37)  | –                        | *No official* `EnsDb`; use `TxDb.Mmusculus.UCSC.mm9.knownGene` |
| Mouse   | mm10 (GRCm38) | v79                      | `EnsDb.Mmusculus.v79`                          |
| Mouse   | mm39 (GRCm39) | v104, v105               | `EnsDb.Mmusculus.v104`, `EnsDb.Mmusculus.v105`  |


### 🧪 Key QC Metrics and Suggested Thresholds (Per Cell/Nucleus)

| **Metric**                 | **Interpretation**                                                    | **Low-Quality Threshold** | **High-Quality Range**   |
|---------------------------|-----------------------------------------------------------------------|--------------------------|-------------------------|
| **Fragments in peaks**      | Proportion of reads in peaks – reflects signal-to-noise                       | < 20–30%               | > 30–50%               |
| **TSS enrichment score**    | Enrichment of reads around transcription start sites                          | < 4–6                  | > 6–10                 |
| **Nucleosome signal**       | Ratio of mono- to di-/tri-nucleosome fragments (chromatin state)              | > 4                    | < 2–3                  |
| **Total fragments**         | Total number of fragments per cell. Avoid very low or very high.              | < 1k or > 50k          | 3k–30k                 |
| **Blacklist ratio**         | Fraction of reads in ENCODE blacklist regions (noise -High = poor quality)    | > 0.01                 | < 0.01                 |
| **Mono-/multi-nucleosome**  | Fragment length distribution should show clear banding        | Poor/no banding     | Visible nucleosome bands     |
| **Doublet score**           | Estimate of multiplets (2+ cells) per barcode (Use to exclude multiplets)     | High                   | Low (filtered out)     |


### Quality control

[Baek and Lee, 2020](https://www.sciencedirect.com/science/article/pii/S2001037020303019#s0015) emphasized the importance of filtering out barcodes corresponding to low-quality cells or doublets after processing sequencing read data. In general, single-cell sequencing QC relies on metrics like read counts (count depth) and feature counts per barcode ([Luecken and Theis, 2019](https://www.scopus.com/record/display.uri?eid=2-s2.0-85067863532&origin=inward&txGid=44862f35717b260b21d411030ddd82fe)). Barcodes with either abnormally low or high read/feature counts are typically flagged as low-quality cells or multiplets, respectively.

However, scATAC-seq data offers additional QC metrics that better reflect chromatin accessibility quality. Commonly used indicators include the fraction of reads in peaks (FRiP), promoter read ratios, blacklist region read ratios, and transcription start site (TSS) enrichment scores ([Buenrostro et al., 2015](https://www.nature.com/articles/nature14590), [Fang et al., 2021](https://www.nature.com/articles/s41467-021-21583-9), [Granja et al., 2021](https://www.nature.com/articles/s41588-021-00790-6)). Barcodes lacking characteristic nucleosome banding patterns—typical of high-quality ATAC-seq data—are also excluded ([Cusanovich et al., 2015](https://www.science.org/doi/epdf/10.1126/science.aab1601?src=getftr&utm_source=sciencedirect_contenthosting&getft_integrator=sciencedirect_contenthosting), [Cusanovich et al., 2018](https://www.cell.com/cell/fulltext/S0092-8674(18)30855-9)). Additionally, peaks located in blacklist regions or overlapping housekeeping genes may be removed during feature filtering ([Fang et al., 2021](https://www.nature.com/articles/s41467-021-21583-9)).

It's important to note that no universal QC thresholds apply to all datasets. QC criteria should be adapted based on specific sample characteristics, such as data complexity, cellular heterogeneity, expected cell types, batch effects, or sequencing platform used ([Baek and Lee, 2020](https://www.sciencedirect.com/science/article/pii/S2001037020303019#s0015)).

For more information on QC and other sc-ATAC-Seq related methods, see [Lei Xiong et al., 2019](https://www.nature.com/articles/s41467-019-12630-7#Sec10), [Li et al., 2021](https://www.nature.com/articles/s41467-021-26530-2), [Stuart et al., 2021](https://www.nature.com/articles/s41592-021-01282-5#Sec9),
[Taavitsainen et al, 2021](https://www.nature.com/articles/s41467-021-25624-1#Sec10), [De Rop et al., 2023](https://www.nature.com/articles/s41587-023-01881-x), [Gamache et al., 2023](https://link.springer.com/article/10.1186/s13578-023-01120-5#Sec10)


#### Post alignment/cell quality filtering parameters

We recommend that the user use the following parameters for initial `scATAC` QC, and then adjust accordingly if necessary:

| Parameter                           | Suggested Value | Corrected Comment                                  |
| ----------------------------------- | -------------- | -------------------------------------------------- |
| `peak_region_fragments_min_upstream` | `"100"`        | Cells with very few fragments in peaks likely represent background noise, empty droplets, or low-quality nuclei.          |
| `pct_reads_in_peaks_value_upstream`  | `"15"`          | % reads in peaks – **> 15%** (or > 20%)     |
| `blacklist_ratio_value_upstream`     | `"0.05"`        | % reads in ENCODE blacklist – **< 0.05**           |
| `mitochondrial_value_upstream`       | `"5"`           | % mitochondrial reads – **< 5%**                   |
| `TSS.enrichment_value_upstream`      | `"10"`          | TSS enrichment score – **> 2** |
| `nucleosome_signal_value_upstream`   | `"4"`           | Nucleosome signal – **< 4**                        |


### (2) Estimating and filtering out doublets

Popular approach of single-cell uses oil droplets or wells to isolate single cells along with barcoded beads. Depending on the cell density loaded, a proportion of reaction volumes (i.e. droplets or wells) will capture more than one cell, forming 'doublets' (or 'multiplets'), i.e. two or more cells captured by a single reaction volume and thus sequenced as a single-cell artifact.

The proportion of doublets is proportional to the number of cells captured. It is common in single-cell experiments to have 10-20% doublets, making accurate doublet detection critical.

Doublets are prevalent in single-cell sequencing data and can lead to artifactual findings. We will use a computational approach to calculate and remove doublets from the library. Here, we use [ScDblFinder](https://bioconductor.org/packages/devel/bioc/vignettes/scDblFinder/inst/doc/scDblFinder.html) method for identifying doublets/multiplets in single-cell data.

The `seurat_obj_raw.rds` object from step (1) is used for this step.
 -  Summary table with doublet metrics and doublets prediction plot are generated.


### (3) Merging filtered data

Next, we merge count matrices from steps (1-2) after filtering out low quality cells and doublets (optional as defined in the `params`). Seurat object and metadata for the library along with UMAP embeddings are saved to be used for downstream analyses.

### (4) Final QC summary report

Lastly, we provide a final QC summary report containing graphs and summary tables across each QC step.

## Folder structure

The structure of this folder is as follows:

```
├── 01A_run_signac_qc.Rmd
├── 01B_run_signac_qc_multiple_samples.R
├── 02_run_scDblFinder.Rmd
├── 03_run_filter_object.Rmd
├── 04_run_summary_report.Rmd
├── plots
├── lsf-script.txt
├── README.md
├── results
├── run-upstream-analysis.R
├── run-upstream-analysis.sh
└── util
|   ├── function-create-UMAP.R
|   ├── function-process-signac.R
|___└── function-run-QC.R
```
````

`upstream-analysis` module

#	steps	aim
1	Signac QC metrics	To infer QC metrics and remove low quality cells

🧪 Key QC Metrics and Suggested Thresholds (Per Cell/Nucleus)

Metric	**Interpretation**	**Low-Quality Threshold**	**High-Quality Range**
Fragments in peaks	Proportion of reads in peaks – reflects signal-to-noise	< 20–30%	> 30–50%
TSS enrichment score	Enrichment of reads around transcription start sites	< 4–6	> 6–10
Nucleosome signal	Ratio of mono- to di-/tri-nucleosome fragments (chromatin state)	> 4	< 2–3
Total fragments	Total number of fragments per cell. Avoid very low or very high.	< 1k or > 50k	3k–30k
Blacklist ratio	Fraction of reads in ENCODE blacklist regions (noise -High = poor quality)	> 0.01	< 0.01
Mono-/multi-nucleosome	Fragment length distribution should show clear banding	Poor/no banding	Visible nucleosome bands
Doublet score	Estimate of multiplets (2+ cells) per barcode (Use to exclude multiplets)	High	Low (filtered out)

`upstream-analysis` module

#	steps	aim
1	Signac QC metrics	To infer QC metrics and remove low quality cells
2	scDblFinder method	To identify doublets/multiplets

```
112 ### (2) Estimating and filtering out doublets
113
114 Popular approach of single-cell uses oil droplets or wells to isolate single cells along with barcoded beads. Depending on the cell density loaded, a proportion of reaction volumes (i.e. droplets or
    wells) will capture more than one cell, forming ‘doublets’ (or ‘multiplets’), i.e. two or more cells captured by a single reaction volume and thus sequenced as a single-cell artifact.
115
116 The proportion of doublets is proportional to the number of cells captured. It is common in single-cell experiments to have 10-20% doublets, making accurate doublet detection critical.
117
118 Doublets are prevalent in single-cell sequencing data and can lead to artifactual findings. We will use a computational approach to calculate and remove doublets from the library. Here, we use
    [ScDblFinder](https://bioconductor.org/packages/devel/bioc/vignettes/scDblFinder/inst/doc/scDblFinder.html) method for identifying doublets/multiplets in single-cell data.
119
120 The `seurat_obj_raw.rds` object from step (1) is used for this step.
121  -  Summary table with doublet metrics and doublets prediction plot are generated.
```

`upstream-analysis` module

#	steps	aim
1	Signac QC metrics	To infer QC metrics and remove low quality cells
2	scDblFinder method	To identify doublets/multiplets (step 2 is optional during final filttering)
3	Merge and filter data	To create clean object for downstream analysis
4	Final QC summary report	To summarize all results in one

```
### (3) Merging filtered data

Next, we merge count matrices from steps (1-2) after filtering out low quality cells and doublets (optional as defined in the `params`). Seurat object and metadata for the library along with UMAP
embeddings are saved to be used for downstream analyses.

### (4) Final QC summary report

Lastly, we provide a final QC summary report containing graphs and summary tables across each QC step.
```

scEpiGenie analysis modules

README.md

Preview on Save | Preview | Run

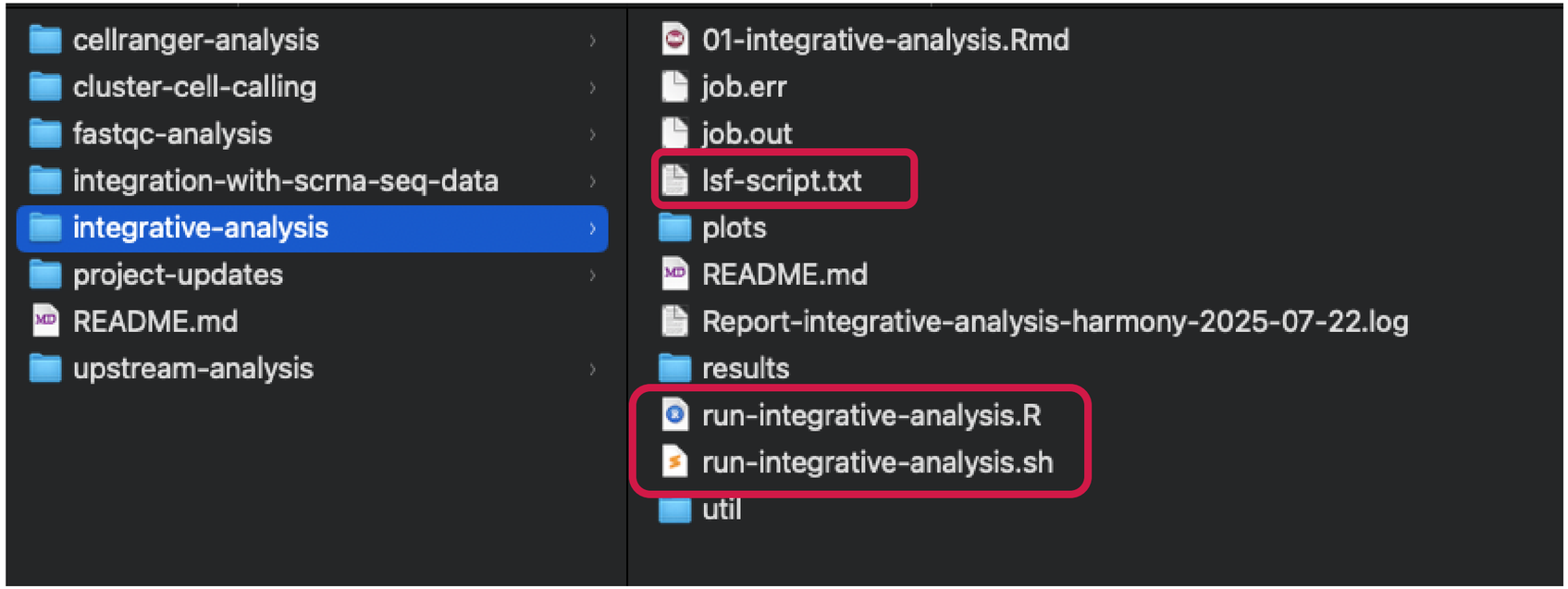
Source | Visual

```
1  # Using Analysis Modules in Single-cell ATAC-seq workflow (scEpiGenie)
2
3  This repository contains a collection of analysis modules designed to process and analyze single-cell ATAC (scATAC-Seq) data from 10X
   sequencing technology.
4
5  **Analysis modules**
6
7  Each module is self-contained and can be executed independently or as part of a larger analysis pipeline. Below is a summary of each
   analysis module, including whether they are required or optional. Furthermore, the analysis modules should be run in the following
   recommended order:
8
9  1. `fastqc-analysis` module (description="Pipeline for FastQC quality control tool for high throughput sequence data analysis.",
   required=True)
10 2. `cellranger-analysis` module (description="Pipeline for running and summarizing Cell Ranger count for single or multiple libraries.",
   required=True)
11 3. `upstream-analysis` module (description="Pipeline for estimating QC metrics and filtering low quality cells.", required=True)
12 4. `integrative-analysis` module (description="Pipeline for Integrative analysis.", required=False)
13 5. `cluster-cell-calling` module (description="Pipeline for cluster cell calling and gene marker analysis.", required=True)
14 6. `integration-with-scrna-seq-data` module (description="Pipeline for integrating scATAC-seq with scRNA-seq data.", required=True)
15 7. `project-updates` module (description="Pipeline for summarizing results from all modules and generating project reports.",
   required=False)
16
```

Downstream

`integrative-analysis` module

description="Pipeline for Integrative analysis.", required=False.



`integrative-analysis` module

README.md

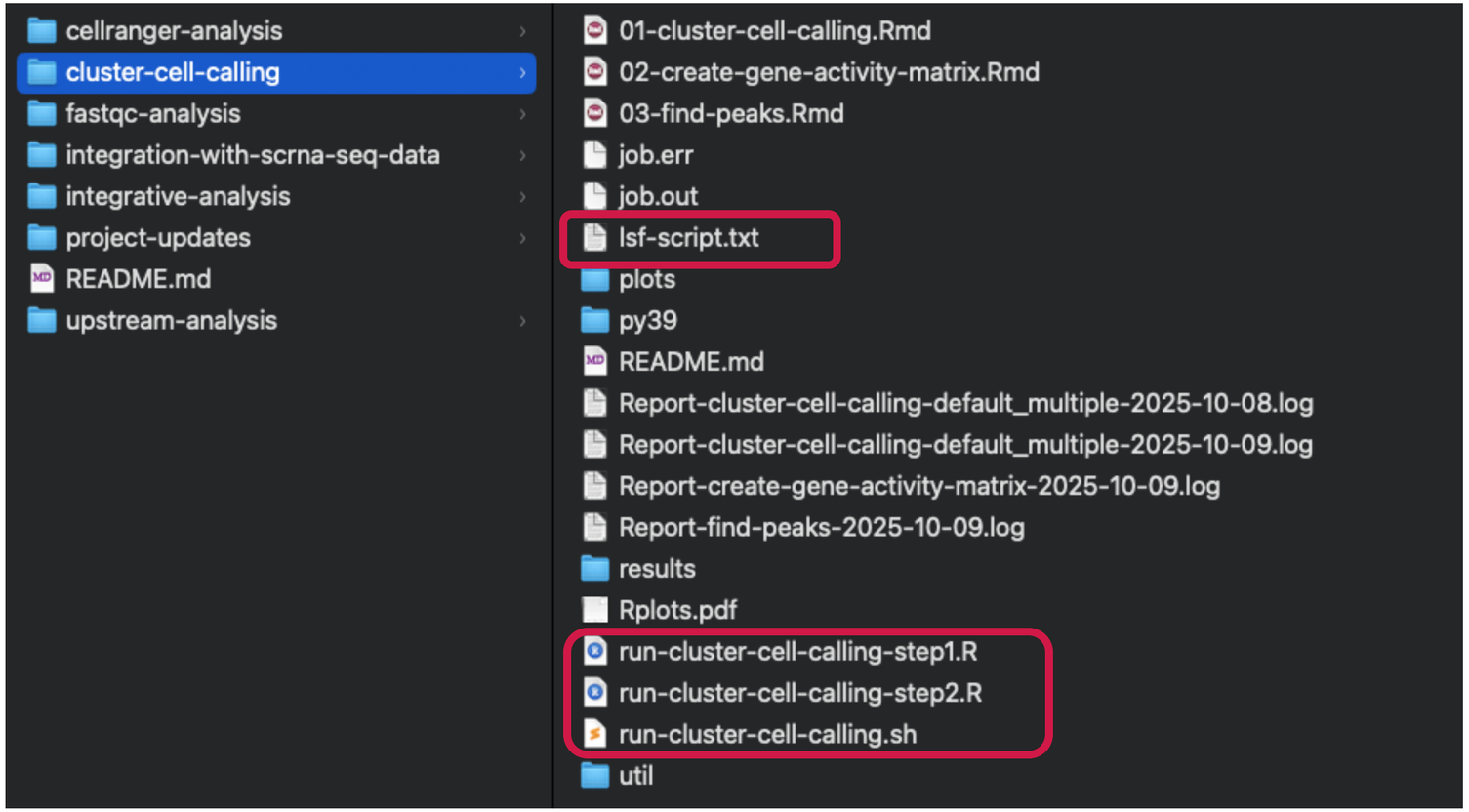
Preview on Save | Preview | Run

Source | Visual

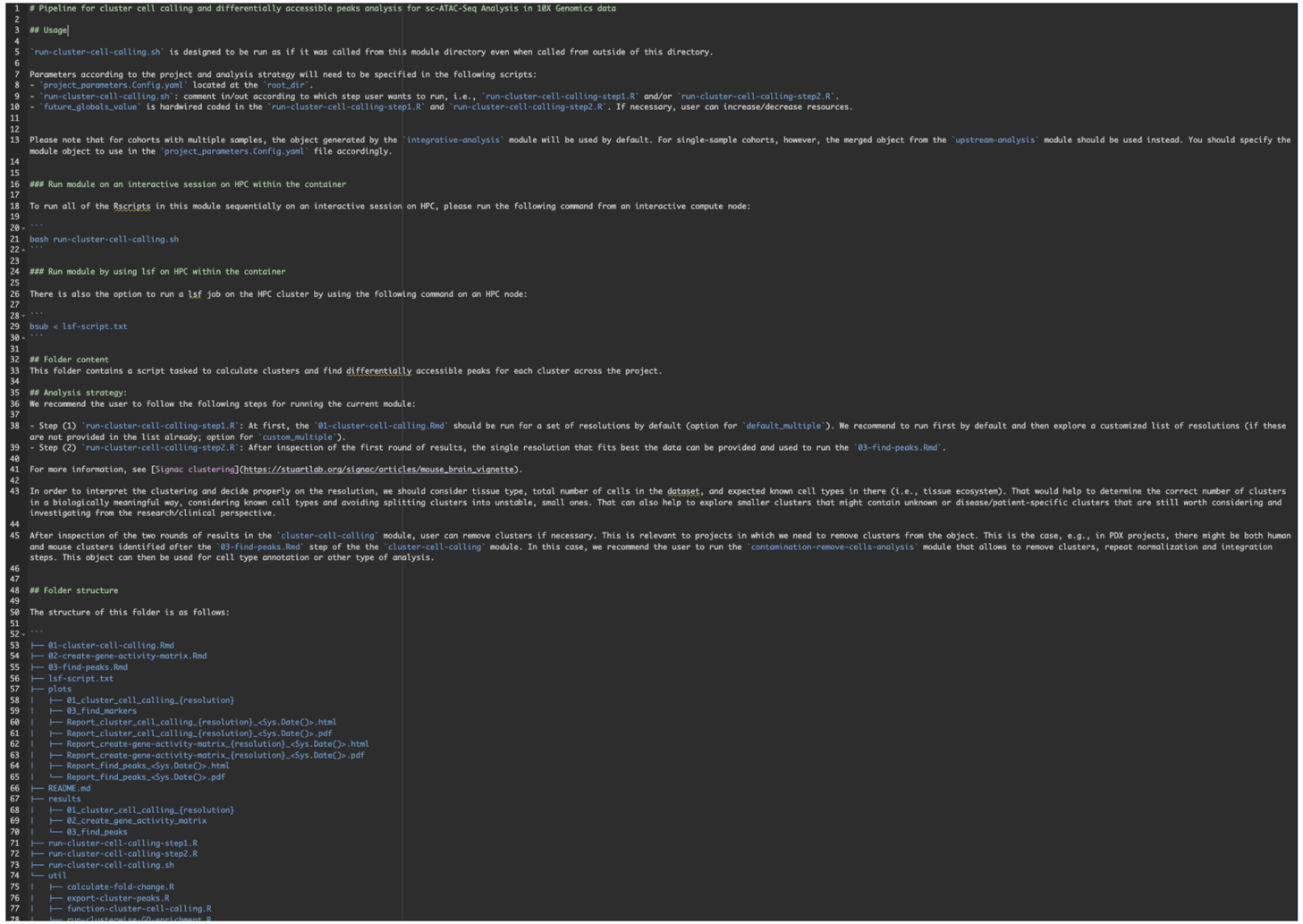
```
# Pipeline for Integrative analysis for sc-ATAC-Seq Analysis in 10X Genomics data

## Usage

`run-integrative-analysis.sh` is designed to be run as if it was called from this module directory even when called from outside of this directory.

Parameters according to the project and analysis strategy will need to be specified in the following scripts:
- `project_parameters.Config.yaml` located at the `root_dir`.
- `future_globals_value` is hardwired coded in the `run-integrative-analysis.R`. If necessary, user can increase/decrease resources.

The `integrative-analysis` module is intended for use with multi-sample cohorts. It should be excluded when analyzing single-sample cohorts, as it relies on data integration across multiple samples.


### Run module on an interactive session on HPC within the container

To run all of the Rscripts in this module sequentially on an interactive session on HPC, please run the following command from an interactive compute node:

```
bash run-integrative-analysis.sh
```

Run module by using lsf on HPC within the container

There is also the option to run a lsf job on the HPC cluster by using the following command on an HPC node:

```
bsub < lsf-script.txt
```


Folder content

This folder contains a script tasked to integrate the samples across the project.

The script named `custom-seurat-functions.R` is from [CellGen Programme Template Notebooks](https://github.com/cellgeni/notebooks).

Folder structure

The structure of this folder is as follows:

```
├── 01-integrative-analysis.Rmd
├── lsf_script.txt
├── plots
|   ├── plots_{integration_method}
|   └── Report-integrative-analysis-{integration_method}-<Sys.Date()>.html
├── README.md
├── results
|   ├── metadata_{integration_method}
|   └── seurat_obj_integrated_{integration_method}.rds
├── run-integrative-analysis.R
├── run-integrative-analysis.sh
└── util
|   ├── custom-seurat-functions.R
|__ └── function-samples-integrate.R
```
```

`cluster-cell-calling` module

description="Pipeline for cluster cell calling and differentially accessible peaks analysis.", required=True.



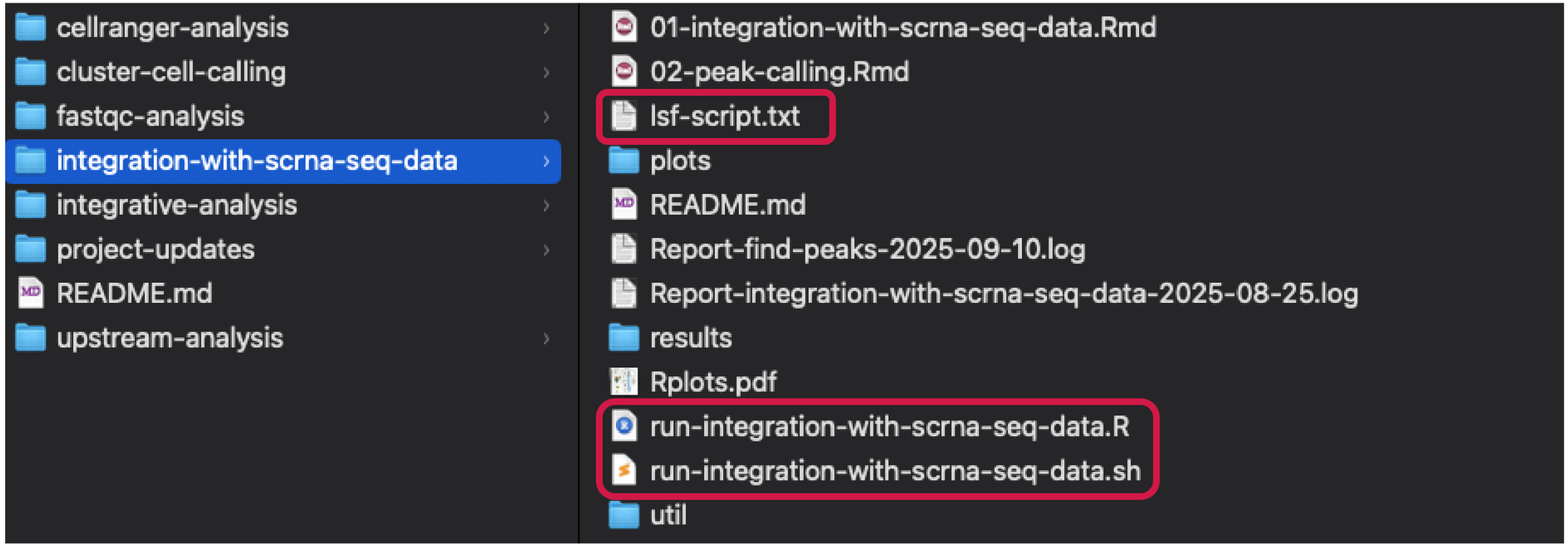
`cluster-cell-calling` module



```
# Pipeline for cluster cell calling and differentially accessible peaks analysis for sc-ATAC-Seq Analysis in 10X Genomics data

## Usage

`run-cluster-cell-calling.sh` is designed to be run as if it was called from this module directory even when called from outside of this directory.

Parameters according to the project and analysis strategy will need to be specified in the following scripts:
- `project_parameters.Config.yaml` located at the `root_dir`.
- `run-cluster-cell-calling.sh`: comment in/out according to which step user wants to run, i.e., `run-cluster-cell-calling-step1.R` and/or `run-cluster-cell-calling-step2.R`.
- `future_globals_value` is hardwired coded in the `run-cluster-cell-calling-step1.R` and `run-cluster-cell-calling-step2.R`. If necessary, user can increase/decrease resources.


Please note that for cohorts with multiple samples, the object generated by the `integrative-analysis` module will be used by default. For single-sample cohorts, however, the merged object from the `upstream-analysis` module should be used instead. You should specify the module object to use in the `project_parameters.Config.yaml` file accordingly.


### Run module on an interactive session on HPC within the container

To run all of the Rscripts in this module sequentially on an interactive session on HPC, please run the following command from an interactive compute node:

```
bash run-cluster-cell-calling.sh
```

Run module by using lsf on HPC within the container

There is also the option to run a lsf job on the HPC cluster by using the following command on an HPC node:

```
bsub < lsf-script.txt
```

Folder content
This folder contains a script tasked to calculate clusters and find differentially accessible peaks for each cluster across the project.

Analysis strategy:
We recommend the user to follow the following steps for running the current module:

- Step (1) `run-cluster-cell-calling-step1.R`: At first, the `01-cluster-cell-calling.Rmd` should be run for a set of resolutions by default (option for `default_multiple`). We recommend to run first by default and then explore a customized list of resolutions (if these are not provided in the list already; option for `custom_multiple`).
- Step (2) `run-cluster-cell-calling-step2.R`: After inspection of the first round of results, the single resolution that fits best the data can be provided and used to run the `03-find-peaks.Rmd`.

For more information, see [Signac clustering](https://stuartlab.org/signac/articles/mouse_brain_vignette).

In order to interpret the clustering and decide properly on the resolution, we should consider tissue type, total number of cells in the dataset, and expected known cell types in there (i.e., tissue ecosystem). That would help to determine the correct number of clusters in a biologically meaningful way, considering known cell types and avoiding splitting clusters into unstable, small ones. That can also help to explore smaller clusters that might contain unknown or disease/patient-specific clusters that are still worth considering and investigating from the research/clinical perspective.

After inspection of the two rounds of results in the `cluster-cell-calling` module, user can remove clusters if necessary. This is relevant to projects in which we need to remove clusters from the object. This is the case, e.g., in PDX projects, there might be both human and mouse clusters identified after the `03-find-peaks.Rmd` step of the the `cluster-cell-calling` module. In this case, we recommend the user to run the `contamination-remove-cells-analysis` module that allows to remove clusters, repeat normalization and integration steps. This object can then be used for cell type annotation or other type of analysis.

Folder structure

The structure of this folder is as follows:

```
├── 01-cluster-cell-calling.Rmd
├── 02-create-gene-activity-matrix.Rmd
├── 03-find-peaks.Rmd
├── lsf-script.txt
├── plots
|   ├── 01_cluster_cell_calling_{resolution}
|   ├── 03_find_markers
|   ├── Report_cluster_cell_calling_{resolution}_<Sys.Date()>.html
|   ├── Report_cluster_cell_calling_{resolution}_<Sys.Date()>.pdf
|   ├── Report_create-gene-activity-matrix_{resolution}_<Sys.Date()>.html
|   ├── Report_create-gene-activity-matrix_{resolution}_<Sys.Date()>.pdf
|   ├── Report_find_peaks_<Sys.Date()>.html
|   └── Report_find_peaks_<Sys.Date()>.pdf
├── README.md
├── results
|   ├── 01_cluster_cell_calling_{resolution}
|   ├── 02_create_gene_activity_matrix
|   └── 03_find_peaks
├── run-cluster-cell-calling-step1.R
├── run-cluster-cell-calling-step2.R
├── run-cluster-cell-calling.sh
└── util
|   ├── calculate-fold-change.R
|   ├── export-cluster-peaks.R
|   ├── function-cluster-cell-calling.R
|   ├── run-clusterprofiler-GO-enrichment.R
```
```

`integration-with-scrna-seq-data` module

description="Pipeline for integrating scATAC-seq with scRNA-seq data.", required=True.



`integration-with-scrna-seq-data` module

README.md

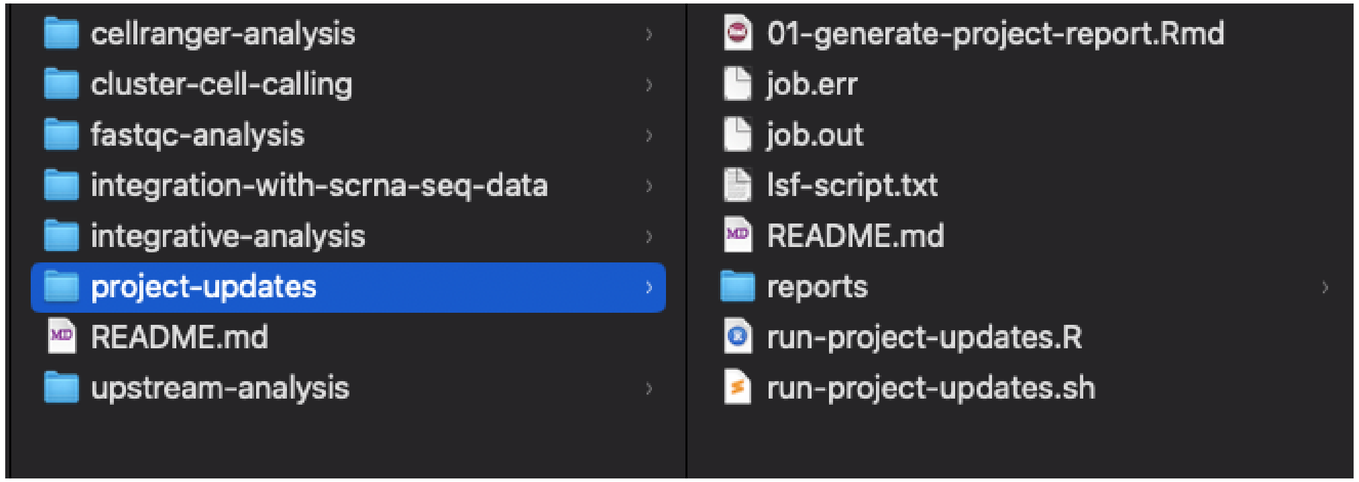
Preview on Save | Preview | Run

Source | Visual

```
# Pipeline for Peak calling per cell type following integration with scRNA-seq data for sc-ATAC-Seq Analysis in 10X Genomics data


## Usage

`run-integration-with-scrna-seq-data.sh` is designed to be run as if it was called from this module directory even when called from outside of this directory.

Parameters according to the project and analysis strategy will need to be specified in the following scripts:
- `project_parameters.Config.yaml` located at the `root_dir`
- `future_globals_value` is hard-coded in each `run-integration-with-scrna-seq-data.R` script for each method. If necessary, user can increase/decrease resources.

We provide a fixed color palette for annotating cell types. Please ensure that the cell type names in your customized cell type marker reference list and/or reference dataset  (if you use any) exactly match those in the `.figures/palettes/cell_types_palette.tsv`. If any cell types are missing, please submit an [issue](https://github.com/stjude-dnb-binfcore/sc-rna-seq-snap/issues) with the list of missing types, and we will add them to the palette. Alternatively, users are welcome to use their own `cell_types_palette.tsv`, as long as the column names (`cell_type_names` and `hex_codes`), file name, and format match those in `./figures/palettes/cell_types_palette.tsv`. We reccomend that users use the same color palette files across pipelines and projects, i.e., same files as for the `sc-rna-seq-snap` pipeline.


### Run module on an interactive session on HPC within the container

To run all of the Rscripts in this module sequentially on an interactive session on HPC, please run the following command from an interactive compute node:

```
bash run-integration-with-scrna-seq-data.sh
```

Run module by using lsf on HPC within the container

There is also the option to run a lsf job on the HPC cluster by using the following command on an HPC node:

```
bsub < lsf-script.txt
```

Folder content

This folder contains a script tasked to perform a data transfer method in the context of scATAC-seq to:

- Classify cells measured with scATAC-seq based on clustering results from scRNA-seq
- Co-embed scATAC-seq and scRNA-seq data

There is also a script to perform peak calling and gene ontology analysis per cell type.

Folder structure

The structure of this folder is as follows:

```
├── 01-integration-with-scrna-seq-data.Rmd
├── 02-peak-calling.Rmd
├── lsf_script.txt
├── plots
|   ├── 01_integration_with_scrna_seq_data
|   ├── 02_peak_calling
|   ├── Report-integration-with-scrna-seq-data-<Sys.Date()>.html
|   └── Report-integration-with-scrna-seq-data-<Sys.Date()>.pdf
|   ├── Report-peak-calling-<Sys.Date()>.html
|   └── Report-peak-calling-<Sys.Date()>.pdf
├── README.md
├── results
|   ├── 01_integration_with_scrna_seq_data
|   └── 02_peak_calling
├── run-integration-with-scrna-seq-data.R
├── run-integration-with-scrna-seq-data.sh
└── util
|   ├── co-embedding-cells.R
|   ├── function_process_fragments.R
|__ └── function-cell-type-fractions.R
```


```

`project-updates` module

description="Pipeline for summarizing results from all modules and generating project reports.", required=False.



Future analysis modules

- Motif analysis with Signac
- Transcription factor footprinting
- Building trajectories with Monocle 3
- Finding co-accessible networks with Cicero

Next steps

✅ **Pipeline development (and testing phase)**

🎉 **Testing phase and code review process**

🚀 **Launching: Beta Release 1.0.0**

🐛 Validation phase by using other datasets/experiments est. by January 2026

Ask me questions!

Tonia
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she/her/hers

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New York, New York

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